Flinders University

DNA Transfer Between Exhibits, Evidence Bags and Workspaces

By

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TABLE OF CONTENTS

THESIS ABSTRACT

Recent advancements in DNA technologies used within forensic science allows genetic information to be gained from smaller amounts of cellular material than was previously possible. With this increase in sensitivity, there is the heightened risk of detecting cellular material unrelated to an alleged event and has been inadvertently introduced into a sample through contamination. The implications of contaminations vary, however these events have the potential to have serious consequences. For this reason, it is essential that best practice in contamination minimisation procedures are used throughout all stages of the handling and processing of forensic evidence.

Forensic laboratories apply rigorous Quality Assurance programs to minimise and detect contamination. Forensic staff contamination can usually be detected through the comparison of all DNA profiles produced within a laboratory to an elimination database that contains the DNA profiles of individuals who are involved in the processing of evidentiary items. The ability to detect contamination by identifying DNA matches with samples from forensic staff through comparison to a laboratory elimination database is limited to the individuals present on the database and does not indicate how and when the transfer occurred. While measures exist to detect contamination by forensic staff, sample-to-sample contamination is more difficult to identify and is therefore more likely to go undetected.

To understand the potential risk for exhibit contamination, it is important to understand the transfer and persistence of DNA, especially within areas where forensic exhibits and samples are processed. It is also essential to identify items which are efficient DNA transfer vectors, and any processes that may facilitate these inadvertent transfer events. Previous research indicates that exhibit packaging can act as an efficient vector for DNA transfer, however before this project, the extent of this transfer, and the contamination risk posed to exhibits, was largely unknown. An overview of previous research which relates to DNA transfer within forensic laboratories and involves exhibit packaging has been provided within Chapter 1.

Within the next chapter (Chapter 2), the levels of DNA which accumulate on the packaging of casework exhibits during routine forensic processing, and specifically throughout the exhibit examination process, was examined. In addition, the source of the accumulating DNA was investigated. Observation of the potential for DNA from an exhibit to accumulate on the exterior of its packaging raised the question of the potential for DNA from the exhibit to be transferred further. This concept was explored within Chapter 3, which investigated the DNA accumulating within forensic workspaces within exhibit storage locations. The question of the potential mechanism involved in the accumulation of exhibit DNA on packaging and the successive transfer of this DNA was addressed within Chapter 4, where DNA transfer between and through the porous surface of paper evidence bags was examined.

This research assists with filling an important knowledge gap within the existing DNA transfer and persistence literature and provides the forensic community with an understanding of the level of exhibit contamination risk posed by DNA that accumulates on exhibit packaging. The data presented within this work demonstrates the potential for exhibit packaging to act as an efficient DNA transfer vector and reinforces the need for police and forensic laboratories to review their current contamination minimisation procedures, to ensure that they are adequate, given the highly sensitive modern DNA profiling technologies.

DECLARATION

I certify that:

1. this thesis does not incorporate material which has been accepted for the award of any other degree or diploma

2. the research within this thesis will not be submitted for any other future degree or diploma without the permission of Flinders University

3. to the best of my knowledge and belief, this thesis does not contain any material previously published or written by another person except where due reference is made in the body of the thesis, and

4. if generative artificial intelligence has been used in my thesis it has been duly acknowledged with details to identify the extent to which generative artificial intelligence formed the final thesis.

Signed: Claire Mercer

Date: 10/09/2025

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LIST OF ABBREVIATIONS

ANZFSS	Australian and New Zealand Forensic Science Society	**pg**	Picogram
bp	Base Pair	**PG**	Probabilistic Genotyping
CE	Capillary Electrophoresis	**POI**	Person of Interest
cm	Centimetre	**PPE**	Personal Protective Equipment
CPI	Combined Probability of Inclusion	**QA**	Quality Assurance
DNA	Deoxyribonucleic Acid	**QC**	Quality Control
EtO	Ethylene Oxide	**qPCR**	Quantitative PCR or Real-Time PCR
FSSA	Forensic Science South Australia	**RMP**	Random Match Probability
IAFS	International Association of Forensic Science	**RFU**	Relative Fluorescence Unit
ISFG	International Society for Forensic Genetics	**SA**	Small Autosomal
LIMS	Laboratory Information Management System	**SAPOL**	South Australia Police
LA	Large autosomal	**SNP**	Single Nucleotide Polymorphism
LR	Likelihood Ratio	**STR**	Short Tandem Repeat
MAC	Maximum Allele Count	**TAC**	Total Allele Count
mL	Millilitre	**TPPR**	Transfer, Persistence, Prevalence and Recovery
ng	Nanogram	**UV**	Ultraviolet
NATA	National Association of Testing Authorities	**µL**	Microlitre
NSW	New South Wales	**VNTR**	Variable Number Tandem Repeat
NoC	Number of Contributors	**VPFSD**	Victoria Police Forensic Services Department
PCR	Polymerase Chain Reaction		

LIST OF FIGURES

LIST OF TABLES

ACHIEVEMENTS

CONFERENCE ATTENDANCE

ORAL PRESENTATIONS

3rd Annual College of Science and Engineering Higher Degree Research Conference, Adelaide, Australia, 2019. *Investigation of the transfer of DNA between exhibits, evidence bags and workspaces.*

22nd Triennial Meeting of International Association of Forensic Sciences in conjunction with the 25th Symposium of the Australian & New Zealand Forensic Science Society, *Sydney, Australia 2020 The DNA Composition on the exterior of casework exhibit bags.* Abstract accepted but conference was cancelled due to COVID-19.

***The Australian and New Zealand Forensic Science Society SA branch* Advances in Forensic Science Research Student Presentations** (online) Adelaide, Australia 2021. *Study of DNA Accumulation and Transfer Within and Operational Forensic Exhibit Storeroom.*

The 9th Crossing Forensic Borders (online) 2021. *The DNA Composition on The Exterior of Evidence Bags.*

Forensic Science SA Seminar Series presentation (online) 2022. *DNA Transfer Between Exhibits, Evidence Bags And Workspaces.*

29th Congress of the International Society for Forensic Genetics, Washington, USA, 2022. *DNA Transfer Between Exhibits, Evidence Bags And Workspaces.*

POSTER PRESENTATIONS

28th Congress of the International Society for Forensic Genetics, Prague, Czech Republic, 2019. *DNA Transfer Between Evidence Bags During Casework.*

22nd Triennial Meeting of International Association of Forensic Sciences in conjunction with the 25th Symposium of the Australian & New Zealand Forensic Science Society, Sydney, Australia 2020. *Study of DNA accumulation and transfer within an operational forensic exhibit storeroom.* Abstract accepted but conference was cancelled due to COVID-19.

25th International Symposium of The Australian and New Zealand Forensic Science Society, Brisbane, Australia 2022. *DNA Transfer Through Evidence Bags.*

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31st International Symposium on Human Identification (virtual) 2020.

7th Annual Human Identification Solutions Conference (virtual) 2021.

8th Annual Human Identification Solutions Conference (virtual) 2022.

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ANZFSS Symposium Award 2020. Awarded $2400 by the South Australian ANZFSS branch to assist with the cost of registration, airfares and accommodation to attend the 22nd Triennial Meeting of the International Association of Forensic Sciences in conjunction with the 25th International Symposium on the Forensic Sciences to be held in Sydney, Australia in May 2021. *NB Award was not received due to conference cancellation.*

Flinders University Student Association Development Grant 2022. A grant of $500 was awarded by the Flinders University Student Association to contribute to the cost of attendance at the 25th International Symposium of the Australia and New Zealand Forensic Science Society in Brisbane, Australia.

College of Science and Engineering 2022 Higher Degree by Research Conference Support. A grant of $500 was awarded by Flinders University as a contribution towards the costs of travel, registration and accommodation associated with attendance at the 25th International Symposium of the Australia and New Zealand Forensic Science Society in Brisbane, Australia.

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Case 1- Candidate performed sampling for trace DNA on letters under investigation by NSW Police.

PEER REVIEWED PUBLICATIONS

Publications awarded during the duration of the candidature, which I have contributed as first author are listed below:

Reference	**Impact factor**	**Citations**	**Year of publication**	**Chapter in thesis**
Claire Mercer, Damien Abarno, Phillippa Hearnden, Adrian Linacre. DNA transfer between evidence bags: is it a means for incidental contamination of items? Australian Journal of Forensic Sciences, 2021. **53**(3): p. 256-270.	1.9	4	2019	6

Claire Mercer, Julianne Henry, Duncan Taylor, Adrian Linacre. What's on the bag? The DNA composition of evidence bags pre- and post-exhibit examination. Forensic Sci Int Genet, 2022. **57**: p. 102652.	4.82	8	2021	2
Claire Mercer, Duncan Taylor, Julianne Henry, Adrian Linacre. DNA accumulation and transfer within an operational forensic exhibit storeroom. Forensic Sci Int Genet, 2023. **62**: p. 102799.	4.82	15	2022	3

LIST OF AUTHOR CONTRIBUTIONS

Title: DNA transfer between evidence bags: is it a means for incidental contamination of items? 2019.

Author	**Participation**
Claire Mercer (Candidate)	Conceptual/experimental design, performed laboratory work and data analysis and prepared the manuscript.
Damien Abarno	Assisted with experimental design, data analysis and manuscript editing.
Phillippa Hearnden	Assisted with experimental design, data analysis and manuscript editing.
Adrian Linacre	Assisted with experimental design, data analysis and manuscript editing.

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Author	**Participation**
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Julianne Henry	Assisted with experimental design, data analysis and manuscript editing.
Duncan Taylor	Aided in statistical analyses, data processing and presentation and manuscript editing.
Adrian Linacre	Assisted with experimental design and data analysis and manuscript editing.

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Author	Participation
Claire Mercer (Candidate)	Conceptual/experimental design, performed laboratory work and data analysis and prepared the manuscript.
Duncan Taylor	Aided in statistical analyses, data processing and presentation and manuscript editing.
Julianne Henry	Assisted with experimental design, data analysis and manuscript editing.
Adrian Linacre	Assisted with experimental design and data analysis and manuscript editing.

CHAPTER 1 : INTRODUCTION

1.1 Preface

The risk of contamination has become more apparent with the improved sensitivity of modern DNA profiling technologies. The increased probability of detecting DNA that is unrelated to an alleged event is a disadvantage that comes with the ability to detect trace amounts of cellular material. With the evolution of DNA technologies, it is essential to review current operational processes to ensure that best practice procedures are implemented within forensic laboratories. The impacts that advancements in DNA technologies have had on the processing of DNA evidence are detailed within this chapter.

This chapter provides a brief overview of the history and current use of DNA technologies within forensic science and outlines the processes which are involved in the transition of evidence containing biological material from the crime scene to presentation of the evidence in court. This chapter also highlights the importance of quality assurance (QA) within forensic science and how QA is maintained by forensic laboratories. A summary has been provided of the literature currently available that relates to DNA transfer and persistence, exhibit packaging and exhibit contamination.

1.2 DNA Profiling from Past to Present

Since its development as 'DNA fingerprinting' in the 1980s, DNA profiling has advanced significantly. Initial DNA fingerprinting methods involved the examination of Variable Number Tandem Repeat (VNTR) regions within the DNA, through Restriction Fragment Length Polymorphism (RFLP) analysis [1, 2]. In this process, restriction enzymes were used to target DNA regions of interest, which were separated using gel electrophoresis [1, 2]. Southern blotting and probe hybridisation techniques were then used to detect variation in the DNA regions at each chromosomal location (locus) analysed [1, 2]. Through the comparison of polymorphic DNA regions in a sample from an unknown source and a reference sample taken from a known person, an individual could be included or excluded as being the source of DNA.

DNA fingerprinting was first used to resolve an immigration dispute, when it was questioned whether a boy was the son or nephew of a woman who immigrated to the United Kingdom with her children [3]. By comparing the DNA fingerprints from the mother, her three undisputed children and the disputed boy, it was determined that the boy had the same father as the other children, and was the woman's son [3]. After the successful application of DNA fingerprinting in the immigration case, it was used to solve a criminal case, which involved the rape and murder of two teenage girls in Leicestershire, UK [4]. The murderer was able to be identified, and it was determined that it was

not the youth who initially confessed to one of the crimes, therefore in addition to convicting the true perpetrator, this technology also exonerated an innocent man.

With the development of other scientific techniques, traditional DNA fingerprinting methods which utilised Southern Blot, radioactive DNA labels and gel electrophoresis were replaced by methods involving Polymerase Chain Reaction (PCR), fluorescent DNA labels and capillary electrophoresis [5]. DNA analysis systems which involved the use of PCR were significantly faster and more sensitive compared to the early methodologies. Once the limitations of using minisatellite regions within DNA analysis were discovered, smaller DNA regions began being utilised instead. The first forensic application of a DNA analysis system which utilised PCR involved the analysis of a Single Nucleotide Polymorphism (SNP) located in the human leukocyte antigen sequence [6-8]. SNPs are the change of a single nucleotide and occur as a result of a DNA replication error. Compared to minisatellites, SNPs are less affected by sample degradation and less sample is required for the analysis. DNA analysis systems which utilised SNPs were soon replaced with systems that involved the analysis of Short Tandem Repeats (STRs), a more polymorphic and therefore more informative region.

As DNA technologies evolved, ‘DNA fingerprinting’ was renamed to ‘DNA profiling’ and was rapidly adopted by many laboratories worldwide [5]. Nowadays, there are many polymorphic regions of DNA which have been identified and are able to be used for human identification, of which STRs are most frequently used within forensic science [9, 10]. It is likely that STRs will continue to be the most prolific regions used within forensic science, due to the massive amount of STR data which comprises DNA databases throughout the world [11].

1.3 Short Tandem Repeats

STRs are areas of highly repetitive sequence, found within the non-coding regions of DNA. They are inherited in a standard Mendelian manner, with the exception of those found on the Y chromosome, are highly polymorphic and known to vary between individuals within the population [10]. These characteristics allow STRs to be used to differentiate individuals, using only their DNA [12]. Through the analysis of STRs from multiple chromosomal locations (loci), an incredibly high level of discrimination between individuals can be achieved [13]. STRs consist of a 2 to 6 base pair (bp) unit, repeated to produce a region of DNA 50 to 300 bp in total length [14]. STRs are short in length compared to the VNTRs used within early DNA profiling methodologies, which allows DNA profiling to be performed on samples that are degraded or contain small amounts of cellular material [10].

With the availability of technology that allows the automation of a number of stages within the DNA analysis process, and multiplex STR profiling kits, the sensitivity and accessibility of DNA profiling

has increased [9, 15]. Nowadays, full STR profiles can be generated from just a few human cells [16], at a low cost and within 90 minutes [17-19].

1.4 Crime Scene to Court Room

There are a series of processes which must occur between the collection of an exhibit at a crime scene and the presentation of the evidentiary findings in the context of a case in court. The exact procedures involved in the processing of evidence are likely to vary between different forensic laboratories around the world, however there are similarities within each stage of the process [20].

1.4.1 Exhibit Collection and Processing

At a crime scene any evidence that may provide information relating to a matter under investigation could be collected. To minimise the risk of contamination by individuals who attend the scene, exhibits should be collected using Personal Protective Equipment (PPE) [14]. To prevent sample degradation, exhibits containing biological material should be packaged in paper evidence bags [20]. Each item recovered should be packaged separately to prevent any cross-contamination between exhibits [14]. To maintain the integrity of the evidence, bags are sealed using tamper-proof tape and labelled appropriately. For each piece of evidence collected at the crime scene, a chain of custody needs to be established, which is a record of all individuals who have handled the item [14]. Without this record being established and maintained, the integrity of an exhibit is lost, as it cannot be excluded that the item has been tampered with or mis-identified.

Depending on the jurisdiction, police or civilian Crime Scene Investigator (CSI) staff may be responsible for attending crime scenes. Regardless of any difference in the individuals who are responsible for the processing of evidence, there are some stages of this process which apply to almost all exhibits collected throughout the world. These stages include exhibit packaging, transport between the crime scene and laboratory, some form of exhibit storage and exhibit examination, which could be of a biological, physical or chemical nature depending on the matter under investigation.

The exhibit handling and movement process in South Australia is outlined to provide relevant background information to subsequent chapters of this thesis, where samples were taken from the exterior packaging of casework exhibits and an exhibit storage location. In South Australia, crime scenes are attended by South Australia Police (SAPOL) staff [21]. While SAPOL has a specialised CSI unit, many crime scenes are attended by patrolling police officers, who may not have as extensive training in sampling and handling biological material, compared to the CSI unit. Items may be packaged correctly, but sealed bags are frequently placed together at the scene or in vehicles when transporting to the laboratory. Transported items are received at the SAPOL evidence desk, where they are receipted by staff and stored within a secure SAPOL exhibit storeroom. Exhibits that require fingerprint analysis are taken to a SAPOL laboratory for

examination and then returned to the SAPOL exhibit storeroom. Exhibits which require additional types of analysis are collected by Forensic Science SA (FSSA) administration staff who transport the items to the FSSA administration department, where a casefile is created, and each item is assigned to the relevant department for examination. Until the item is collected for examination, it is stored in compactus unit shelving within the secure FSSA exhibit storeroom. Multiple packaged exhibits, from different cases, can be stored together on shelving within the storeroom. Bags are removed by examiners and transferred to evidence recovery units. Once the exhibit examination is complete, the item is returned to the FSSA exhibit storeroom where it may remain for a prolonged period, or be transported back to the SAPOL exhibit storeroom, depending on the case. Occasionally, exhibits are submitted for a second examination. All exhibit movements are tracked within FSSA's electronic Laboratory Information Management System (LIMS).

1.4.2 Exhibit Examination

Exhibits that are being examined for biological material are taken to designated 'DNA-free' laboratories for examination. To minimise the risk of exhibit contamination, full PPE must always be used within examination laboratories: including a face mask, hair net, disposable laboratory coat and gloves. These laboratories also have strict cleaning schedules, and the levels of environmental DNA are monitored to ensure that the cleaning procedures are effective [21].
At the beginning of the examination, the exhibit is carefully removed from its package in a way which ensures no contact is made between the exterior of the bag and the evidence. As PPE is not required during the handling of evidence bags prior to examination, the bags are not considered to be clean. To prevent contamination, the package from which the exhibit was removed is placed away from the exhibit until the examination is complete. Gloves are also changed between handling the evidence packaging and the exhibit. Exhibits are searched for traces of DNA or biological fluids, depending on the nature of the matter under investigation. Samples are taken from areas of interest on the exhibit, using various methods which depend on the item. Generally, swabs are used to sample non-porous surfaces and tapelifts are applied on porous surfaces. These samples are then submitted for DNA analysis. Once the examination is complete, the item is placed back into the evidence bag, which is sealed using tamper-proof tape, and returned to an exhibit storeroom.

1.4.3 Sample Processing

There are several stages involved in the DNA analysis process. The standard DNA profiling workflow consists of: DNA extraction; quantification of the DNA template present in the extract; amplification of the target DNA regions; and the separation of the amplified DNA fragments, typically using capillary electrophoresis (CE). Each of these stages consist of numerous individual steps, which are required to produce the highest quality DNA profile possible.

1.4.3.1 DNA Extraction

DNA extraction is the first stage of the DNA analysis process. To maximise the yield of DNA which can be profiled, the extraction step removes sample impurities and inhibitors which may affect downstream processes. The DNA extraction methods which can be used depend on the type of sample being processed. The DNA IQ™ System (Promega Corporation) is one method which can be used to isolate pure DNA from multiple sample types and was therefore used to extract DNA from all samples in this project. This method involves the addition of a lysis buffer to the sample, which disrupts cellular membranes and releases cellular DNA [22]. The DNA solution is then added to a paramagnetic resin which retains the DNA, while impurities and inhibitors are removed through the addition of wash solutions [22]. The purified DNA is then removed from the resin and resuspended into solution, using an elution buffer. The resultant DNA solution is ready for use in downstream STR analysis processes [22].

1.4.3.2 DNA Quantification

To give the best chance of producing the highest quality DNA profile, the optimal amount of DNA needs to be added to downstream profiling reactions. For this reason, DNA quantification is the next stage of the analysis process. There are several methods which can be used to quantify DNA, each have varying levels of accuracy. In this project the Quantifiler™ Trio DNA Quantification Kit (Applied Biosystems™) was used in conjunction with quantitative PCR (qPCR) technology to quantify DNA in extracted samples. This kit quantifies the total amount of amplifiable human (i.e., male and female) and human male DNA within a sample simultaneously [23]. As each DNA amplification cycle occurs, fluorescent signals are released from probes with reporter dyes attached (Figure 1.1), which allows accurate measurement of the DNA present.

There are multiple regions within the DNA targeted by the Taqman® probes used within this kit [23]. A small autosomal (SA) region is targeted, which produces amplicons of 80 bp, and allows for the detection of DNA in degraded samples. The large autosomal (LA) target produces amplicons of 214 bp and is compared with the SA target to determine whether the sample contains degraded DNA. The amount of male DNA within a sample is measured using a target on the Y chromosome, which produces a 75 bp amplicon [23]. The Quantifiler™ Trio kit also determines whether there are any PCR inhibitors present within the sample [23].

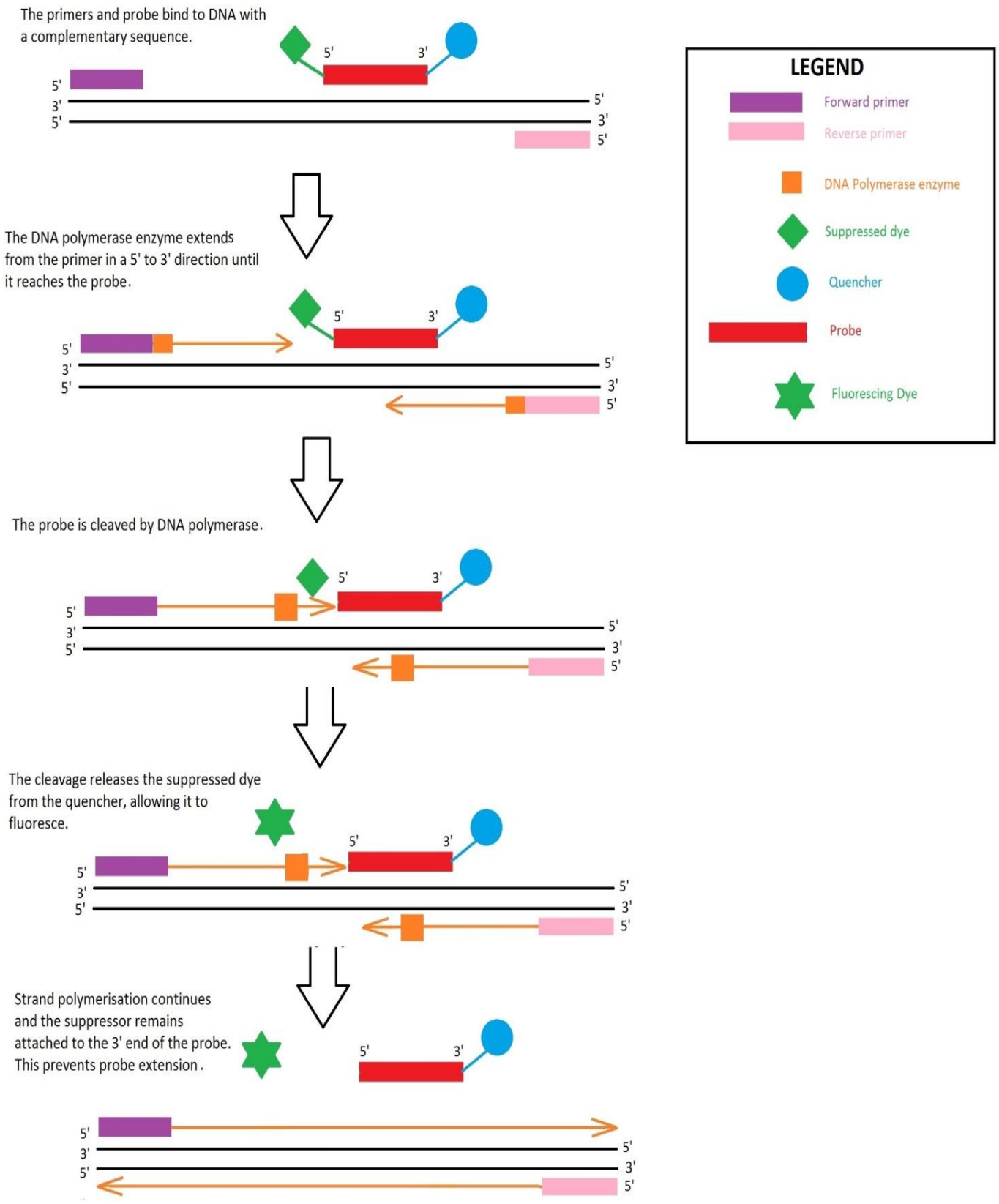

Figure 1.1. Overview of the nuclease assay process which occurs within each PCR cycle, using the Quantifiler™ Trio DNA Quantification Kit. Different fluorescent dye and non-fluorescent quencher combinations are used for each of the target regions within this kit.

1.4.3.3 DNA Amplification

To produce a DNA profile from the extracted and quantified DNA sample, STR regions of interest within the DNA need to be isolated and amplified. This is performed using a PCR based system, which enables the process to be carried out using only a small amount of DNA. There are numerous kits which are currently commercially available and allow DNA from multiple STR regions to be amplified simultaneously. Within this project, the GlobalFiler™ PCR Amplification Kit (Applied Biosystems™) was used. This kit amplifies DNA from 21 autosomal STR regions, one STR region on the Y chromosome, one insertion/deletion region on the Y chromosome and a sex determining marker called Amelogenin [24]. There are six fluorescent dyes within this kit which allow differentiation of the products with overlapping size ranges (in base pairs) from the 24 amplified regions [24]. A matrix file is used to correct the spectral overlap that occurs between the different dyes, which enables the accurate separation of the signal from each dye.

1.4.3.4 Capillary Electrophoresis

To produce a DNA profile from the amplified PCR products, DNA fragments need to be resolved. This process is most commonly performed in forensic STR analysis using CE [25]. The fluorescently labelled DNA fragments are separated by size as they travel along a capillary which contains an anode buffer [26]. A negatively charged voltage is applied to the capillary array header, which creates an electric field through interaction with the anode buffer [26]. This electric field moves the negatively charged DNA fragments through a capillary column to a laser beam which is directed into both sides at the end of the capillaries. Once the DNA fragments reach the laser, the attached fluorescent dyes emit fluorescent light, which is detected by a charged-couple device camera [26]. The instrument converts the detected signal into information which can be used to produce an electropherogram. There is a variety of software currently available that allows the visualisation of electropherograms produced by CE instruments [27] including GeneMapper® ID-X [28], FaSTR™ [29, 30], GeneMarker® HID [31] and OSIRIS [32].

1.4.4 Electropherogram Interpretation

The complexity involved in the interpretation of an electropherogram largely depends on the nature of the DNA sample. PCR inhibition, DNA degradation and the amount of DNA present within a sample are all factors that will influence the quality of the resultant electropherogram. In addition to the quality of an electropherogram produced, the success of interpretation depends on the ability to differentiate allelic and artefactual peaks and accurately assess numbers of profile contributors.

1.4.4.1 Artefacts

Artefactual peaks, which do not represent true alleles, are commonly found within electropherograms and can have a variety of origins. Some of the artefacts that are known to occur have an unknown origin, while the cause of many others is known. Details of commonly observed

artefacts for various STR profiling kits can usually be located within validation studies and technical notes published by the kit manufacturers. Some artefacts that are commonly observed within electropherograms include: stutter, pull-up, split peaks, spikes, dye blobs and n-1 peaks. The artefacts most commonly observed within DNA profiles generated from samples taken within this research were stutter and pull-up.

1.4.4.1.1 Stutter

Stutter peaks are produced as a result of DNA strand slippage during the PCR amplification process. The rate of stuttering is proportional to the number of repeating units within an STR. It has been shown that longest uninterrupted sequence (LUS), which is the longest portion of the repetition of the same sequence within an allele, is a better predictor of the stutter proportions than allele length [33, 34].

Slippage within the template DNA strand results in backwards stutter, which produces a peak one repeating unit less than the true allele peak, as displayed in Figure 1.2. Back stutter is the most predominantly observed stutter variant. Forward stutter produces a peak which is one repeating unit greater than the true allele peak and occurs when strand slippage occurs within the strand of DNA which is being synthesised. Other stutter variants that are known to occur, include double-back stutter, which produces a peak two repeating units less than the true allele, and half-back stutter, which produces a peak half a repeat unit less than the true allele.

The configuration of the repeating unit within an STR influences the frequency of stutter, with di- and trinucleotide repeats being more prone to stutter than more complex repeat units. As stutter is a PCR by-product, it is impacted by the number of PCR cycles and the DNA amplification kit used. Stutter thresholds for each locus vary and different laboratories generally have their own thresholds for stutter products for each kit. Such thresholds can be used in conjunction with other features of a DNA profile, to assist with identifying whether a peak is likely to be artefactual or allelic.

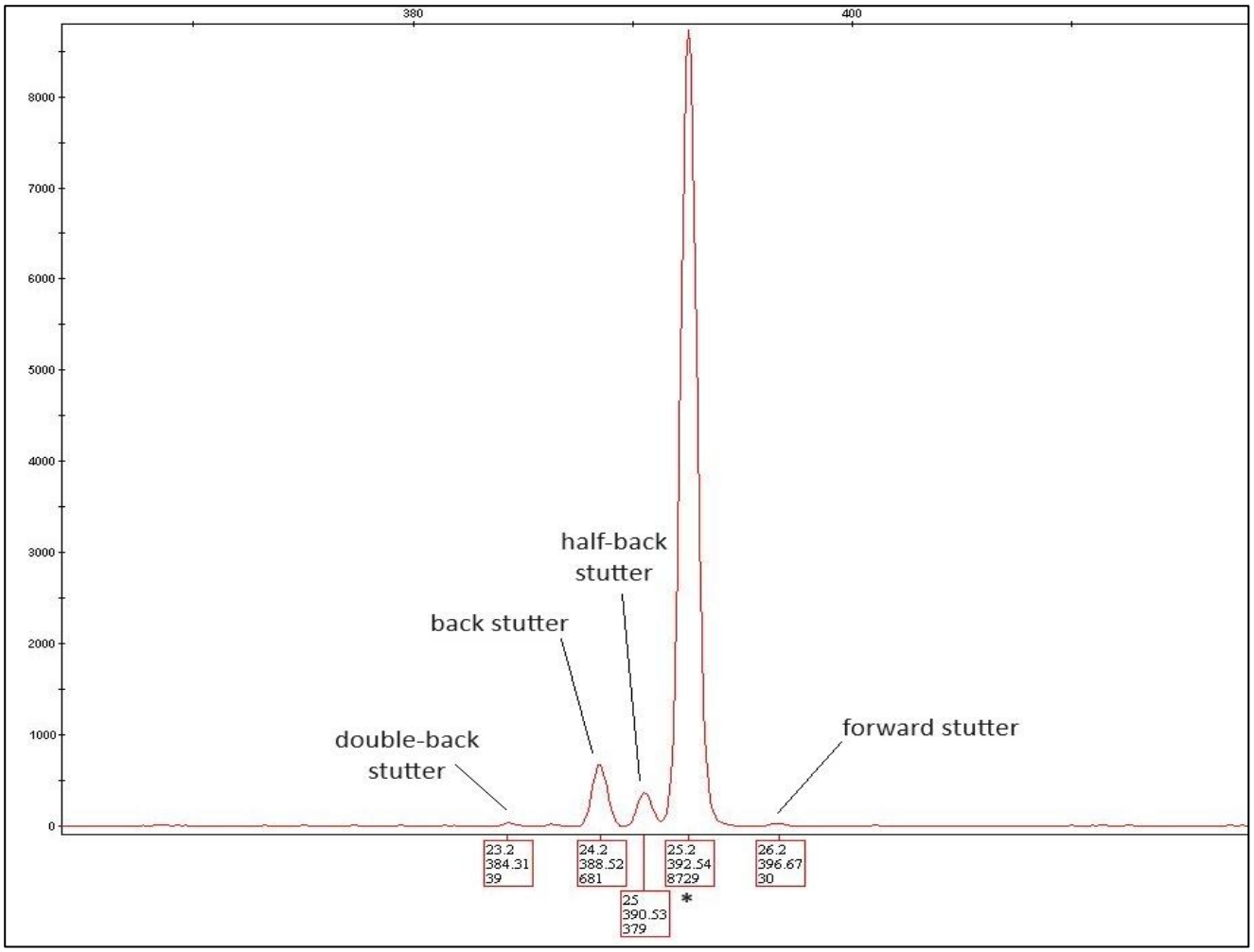

Figure 1.2. Examples of a variety of stutter types that are known to occur within DNA profiles. The allele peak is indicated by an asterisk positioned below the peak and each of the stutter variants are labelled.

1.4.4.1.2 Pull-up

The spectral overlap between the different fluorescent dyes used within STR amplification kits is resolved by the DNA profile analysis software, to produce peaks of a single colour within an electropherogram. Pull-up peaks occur when there is a strong enough fluorescent signal produced by an amplification product, that “bleed through” of fluorescence dye signal occurs and the spectral overlap between dyes is not resolved. Such peaks are observed at the same base pair position as the amplification fragment which produced the intense dye signal, but within a different dye channel, as displayed in Figure 1.3. Pull-up peaks can also occur when a poor-quality matrix file is used to resolve the fluorescent dyes.

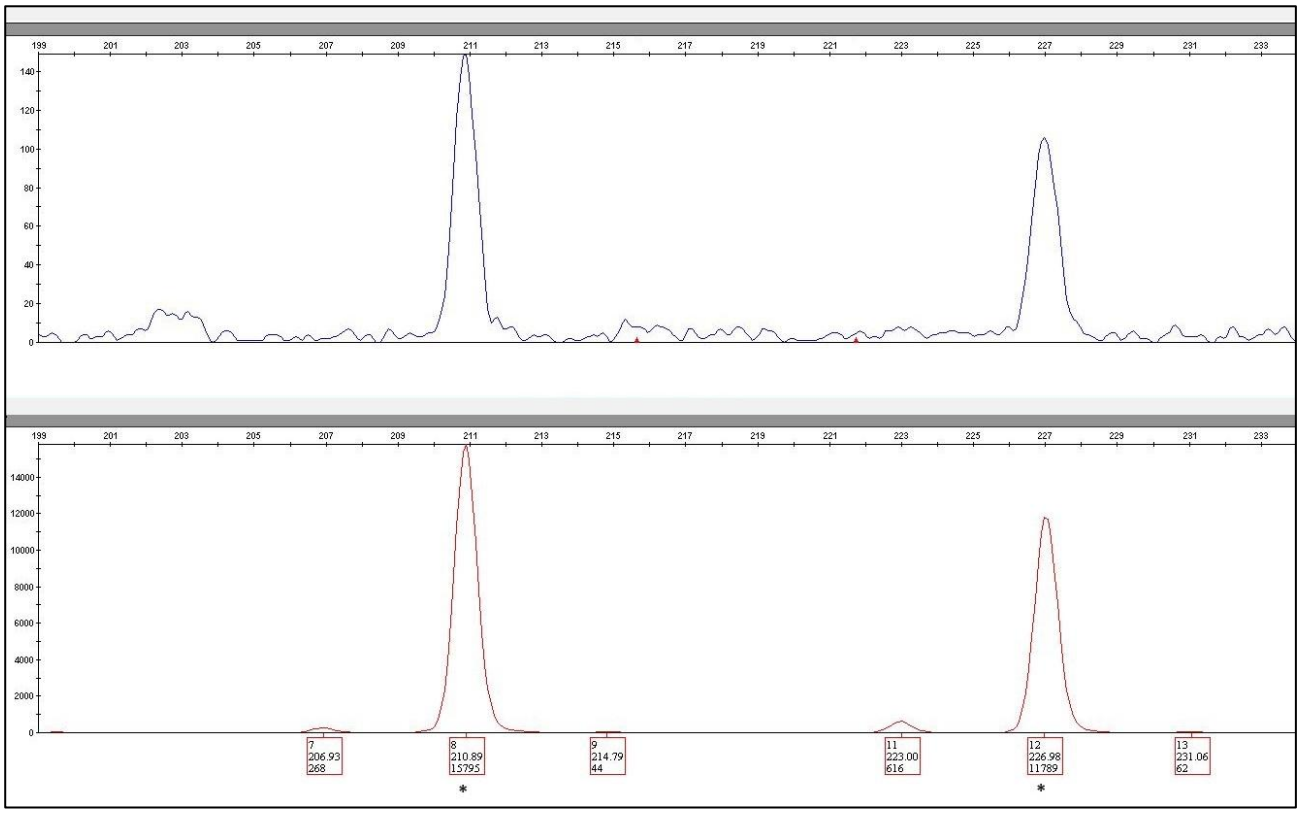

Figure 1.3. Example of two instances of pull-up, where the fluorescent dye signal from the allele peaks (indicated by an asterisk positioned below the peak) within the red dye channel (bottom), has 'bled' into the blue dye channel (top). The pull-up peaks are located at the same base pair position as the corresponding allele peaks.

1.4.4.2 Assessing Number of Profile Contributors

An important stage of the electropherogram interpretation process involves assigning number of contributors (NoC). Assignment of NoC is necessary to carry out mixture interpretation and is required in most downstream DNA profile analysis software [35, 36]. There are a number of different methods that have been previously described to assign NoC, which include Maximum Allele Count (MAC), Total Allele Count (TAC) and likelihood-based approaches [37-40]. Additionally, there are now also a number of tools that can be used to estimate NoC, using various methods [37, 41-45]. Regardless of which NoC assignment method is used, the performance of these methods largely depends on the complexity and quality of the electropherogram [46-48]. Factors which can impact the accuracy of NoC assignment include DNA template, allelic drop-out and drop-in, unremoved artefacts and multiple DNA contributors, especially in cases where there is significant allele sharing between donors [39, 48-50].

Throughout all sections of this research, NoC was assigned to DNA profiles using a combination of MAC and taking into consideration peak height proportions. Compared to using MAC alone, this method is likely to have provided a better representation of the true number of contributors for

many of the samples which yielded complex mixed DNA profiles, due to the instances of allele sharing between contributors.

1.4.5 DNA Profile Interpretation and Analysis

Once the electropherogram interpretation process is complete, the resultant DNA profile is ready for use in downstream comparison. To determine the source of DNA from an STR profile yielded from a crime scene sample, comparison with a DNA profile from a known source needs to occur. In cases where there is a POI whose reference DNA profile (or a sample that can reliably yield a reference profile) is available, a comparison between the reference and evidential DNA profile is performed. In cases where an unknown offender is involved and there is no POI, a match between a DNA profile from a criminal DNA database is usually relied upon to identify the source of the DNA.

When dealing with high quality, single-source DNA profiles, profile comparison is usually relatively straight forward. This contrasts with dealing with mixed DNA profiles, or profiles which have originated from samples of low DNA quality or quantity. With the increased sensitivity of modern DNA profiling technologies, DNA profile interpretation has become more complex [51]. This is partly because the ability to detect such small amounts of DNA has resulted in an increased volume of trace DNA samples being submitted to forensic laboratories for DNA analysis [52]. Trace samples commonly yield poor quality profiles, as they are usually of low DNA quality and quantity and are therefore highly susceptible to allele drop-out and other stochastic effects involved with amplifying low amounts of DNA [51, 53, 54]. With the ability to detect smaller amounts of DNA, there is also the increased possibility of observing drop-in and DNA mixtures. As the NoC within a profile increases, the number of possible allele combinations considered for each DNA donor significantly increases. Due to these factors, the level of complexity involved in interpreting DNA mixtures often exceeds the point of it being reasonable, or in some cases possible, for the mixture interpretation process to be performed by a human DNA analyst. With the development of Probabilistic Genotyping (PG) software, these complex mixtures are now able to be analysed. Within PG, biological modelling is combined with statistical theory to infer genotypes for donors within a DNA profile. There are many variants of PG software available, which are widely used within the forensic biology community and allow the interpretation and analysis of complex DNA profiles.

Historically, DNA profile interpretation was carried out using a binary approach, where the probability of obtaining the evidence for a given genotype is either assigned a value of zero or one, depending on whether the evidence can be accounted for by the genotype. Using the application of rules or thresholds, the possible genotype combinations for a profile contributor are determined. Within binary methods, all combinations of genotypes are considered equally likely and drop-out and drop-in are not considered. Limitations of using the binary methods, which include the inability

to effectively interpret complex mixtures or low-level profiles, led to the development of PG software. PG software can be classified within two categories: semi continuous and fully continuous. Semi-continuous methods can accommodate drop out and drop in but do not model artefacts, such as stutter, or consider peak heights when generating potential genotype sets. Consideration of peak height information is essential when assessing several parameters, which include NoC and the probability of drop out, therefore one limitation of semi-continuous methods is not utilising this information.

Fully continuous models use all quantitative information within a DNA profile to calculate the probability of peak heights for all genotype combinations for each profile contributor. There are many methods that exist, which implement different distributions to model allelic peaks, and in some cases stutter peaks too. In these models, the probability of obtaining a set of peaks heights is determined using assumptions made about the underlying behaviours of peak heights. Continuous methods therefore utilise more information from within a profile than semi-continuous methods.

Within all sections of this research, the continuous PG software STRmix™ was used to analyse and compare DNA profiles and perform Likelihood Ratio (LR) calculations.

1.4.5.1 STRmix™ Software

STRmix™ is one example of a PG software, which employs a fully continuous model to interpret and resolve DNA profiles. This software has been widely validated and implemented in forensic DNA laboratories worldwide. Within STRmix™, the quantitative information from an electropherogram is used to interpret mixtures and calculate the probability of obtaining the DNA evidence given a set of competing hypotheses.

1.4.6 Evaluation of DNA Evidence in Court

The presentation and explanation of evidential findings within a court of law is usually the final stage of the forensic process. The evaluation of DNA evidence within the court room typically relates to providing information about the ‘who’ and ‘how’. The means of addressing the ‘who’ will be discussed first.

1.4.6.1 Propositions and Likelihood Ratios

Traditionally, if the same alleles are identified within a reference profile from a person of interest (POI) and the DNA profile generated from an evidential DNA profile, the statistical weighting of the match must be determined. There are a number of different methods that are currently used by laboratories around the world to calculate the match statistic. The approach taken by each of these methods are either considered as an ‘exclusionary’ or an ‘inferred genotype’ approach. The cumulative probability of inclusion (CPI) or random man not excluded (RMNE) method utilises the

former approach and considers the probability that a random person would be included or excluded as a contributor to the DNA profile. Two methods which use an 'inferred genotype' approach include the use of a random match probability (RMP) and likelihood ratio (LR). The RMP is an estimation of the probability of observing a given DNA profile within a population. The LR is a ratio of probabilities given two or more mutually exclusive propositions and can be expressed as the following equation:

$$LR = \frac{\Pr(E|H_1)}{\Pr(E|H_2)}$$

where E is the evidence (DNA profile) and H_1 and H_2 refer to the two propositions that are being considered. For ambiguous profiles, where it is considered that drop-out or drop-in is likely, utilising an LR has significant advantages over binary approaches where the only conclusions are the DNA profile does or does not match. The use of an LR has become the recommended approach within the field of forensic DNA interpretation [55]. There are a range of mathematical modelling software that can be used to calculate LRs using a range of different propositions, including whether individuals are related or unrelated. When considering a POI as the source of a DNA profile, the following propositions are generally considered:

H1) the POI and (N-1) unrelated individuals are the sources of DNA

H2) N unknown individuals, unrelated to the POI are the sources of DNA

Where N is the total number of profile contributors.

H_1, sometimes referred to as the prosecution hypothesis, is the proposition that a POI is a contributor to the DNA profile. H_2, sometimes referred to as the defence hypothesis, is the proposition that another random individual, who is unrelated to the POI, is the contributor to the DNA profile. When considering an unambiguous single source profile, where all loci match the reference profile of a POI, the probability of the evidence given H1 is equal to 1. The probability that someone other than the POI is the source of the DNA is equal to the RMP. Therefore, for an unambiguous single source profile, the LR in its simplest form is $\frac{1}{RMP}$. While an LR assists with addressing the source of DNA within a sample, it does not provide an indication of how the DNA has been transferred.

1.4.6.2 Distinguishing DNA Transfer Events

The detection of an individual's DNA within a sample does not provide an explanation of the DNA transfer mechanisms involved. Nowadays, questions in court relating to the source of DNA are often superseded by those relating to how the DNA was transferred. To address questions regarding the possible mechanisms involved in DNA transfer, research and data within the

literature is vital. While it is important to recognise that DNA transfer research cannot provide a definitive answer on how DNA has been transferred to an item, this research helps inform how likely certain types of transfers are under specific conditions. This assists forensic scientists in offering expert opinions about the plausibility of certain scenarios. More recently, data from DNA transfer research has also been used to inform statical models such as Bayesian networks where different transfer scenarios are assigned probabilities based on the data input [56-58].

1.5 DNA Transfer and Persistence

1.5.1 DNA Transfer Mechanisms

DNA can be transferred to surfaces through direct and indirect transfer mechanisms. Direct transfer mechanisms involve an individual directly contacting a surface or speaking, coughing or sneezing in close proximity to it [59-64]. Indirect transfer mechanisms involve an individual depositing their DNA onto a surface, which is termed primary transfer, and then the DNA from the initial transfer event is transferred through contact between the surface and another substrate, which is known as secondary transfer [61-68]. Tertiary and quaternary DNA transfer events are also possible with subsequent contacts between substrates, which result in transfer of the DNA from the initial contact event [62-64, 68]. With each transfer event, the amount of DNA transferred (or likelihood of transfer) diminishes.

1.5.2 Factors that Influence DNA Deposition

There are several factors that have been shown to influence the amount of DNA deposited onto a surface [26-31, 34]. The origin of the biological material has been previously shown to influence DNA deposited with biological fluids, such as blood and saliva, generally containing large quantities of undamaged cells and therefore, generally higher amounts of DNA [69]. Compared to biological fluids, less DNA is deposited by touch (‘touch DNA samples’) which is thought to be due to the lack of DNA-rich cellular material and mostly degraded cellular composition [70].

Studies have shown that an individual’s characteristics, such as age, sex, skin conditions and ‘shedder status’ (which is a measure of an individual’s propensity to deposit DNA) can influence the amount of DNA deposited by touch [28-31, 34, 35]. Very young children have been shown to deposit more DNA than other age groups, whereas people over 70 have been shown to generally deposit less [71]. This observation was supported in another study, which only investigated DNA transferred to a surface by males, where it was determined that younger males generally deposited higher amounts of DNA [72].

Some studies suggest that males generally deposit higher quantities of DNA onto a surface through touch compared to females [73, 74], but other studies indicate that there is little difference between sexes [75, 76]. Skin conditions which may cause skin to become flaky, such as dermatitis

or psoriasis, and extremely dry skin, has also been observed to increase the amount of DNA deposited [61, 77, 78].

Some studies have observed that certain individuals consistently deposit higher or lower amounts of DNA than others, allowing them to be classified as a high or low shedder [61, 75, 76, 79-81]. More recently there was an expansion of shedder types to include 'intermediate shedders' [82-86]. Other studies have disputed the concept of shedder status, where quantities of DNA deposited by the same individuals were observed to vary on different days [72, 87].

Variation in the quantities of touch DNA deposited from different areas of the hands and body have also been observed [70, 78]. Higher amounts of DNA were generally deposited in contacts with sebaceous skin areas [70, 88]. Activities conducted before touching a surface have also been shown to vary the amount of DNA deposited. Wearing gloves, handwashing and touching clean objects have been shown to reduce DNA on hands, and therefore also reduced the amount of DNA deposited after these activities [76, 81, 89]. In comparison, activities which increase the amount of DNA on hands such as sweating, or touching items repeatedly that are not DNA-free, can increase the amount of DNA deposited [74, 88, 90].

The type of contact, surrounding conditions, and surface type have also been shown to influence the amount of DNA that will be transferred onto a surface [25]. Higher amounts of DNA are generally transferred to a surface in contacts involving friction and pressure [66, 67, 91]. In some studies, the amount of DNA transferred to a surface increased with the time of contact [92]. However, in other studies, it was determined that contact time had no effect on the amount of DNA deposited, which indicates that majority of DNA is transferred to a surface at the initial time of contact [61]. Moisture has been observed to significantly increase the amount of DNA transferred to a surface [66, 88]. Additionally, more biological material has also been observed to transfer from hard, non-porous substrates to soft, porous surfaces [66, 67, 75, 93, 94].

1.5.3 Factors that Influence DNA Persistence

The persistence of DNA deposited onto a surface also depends on several factors. With an increase in time after sample deposition, the quality of DNA has been shown to decrease [95, 96]. Studies which show variation in the amount of DNA recovered from plastic and cotton, sampled at the same time after DNA deposition, indicate that the surface type may influence the persistence of DNA [67]. High temperatures, ultra-violet (UV) light [97, 98], moisture and microbial activity have also been shown to degrade DNA, therefore exposure to these conditions may decrease the persistence of DNA [95, 96]. Contact between a surface which contains DNA, and another substrate, will also alter the composition of the material present, and may result in the removal or addition of DNA [93].

1.6 Quality Assurance in Forensic Science

Quality Assurance (QA) is another vital component of forensic science, which has significantly evolved since the early origins of DNA profiling. To ensure that forensic laboratories comply with appropriate standards and accurately supply results, they have been compelled to develop QA programs [99]. These programs address the necessary actions to give confidence in the accuracy of the results produced by a forensic laboratory. They ensure that forensic laboratory processes are operating within acceptable limits and that this high standard of operation is consistently maintained. The ongoing processes that are utilised to maintain this high standard are known as Quality Control (QC). Laboratory accreditation is one of many components that adhere to QA within forensic science.

Throughout the world there are a number of organisations that ensure forensic laboratories meet the required QA standards [100]. In Australia this is the National Association of Testing Authorities (NATA). If a forensic laboratory meets the required standards, these organisations will provide accreditation, which recognises that the laboratory can consistently provide reliable results [100]. To ensure that forensic laboratories remain compliant with accreditation standards, their performance is monitored at regular intervals [100].

While there are several components that contribute to QA and QC in Forensic Science, one vital aspect is the implementation of procedures that minimise the risk of contamination and allow the detection of any events, if and when they occur.

1.6.1 Contamination Minimisation

From publicised cases which involved the miscarriage of justice, inadmissibility of evidence and erroneous police investigative leads, the forensic community is aware of the potential consequences of contamination incidents [101-103]. Contaminations can occur throughout any stage of the handling and processing of evidence and can have serious consequences for criminal investigations [103-105]. DNA from contaminations can mask alleles within a DNA profile generated from a crime scene sample and produce mixed DNA profiles that reduce the evidential value of any matches obtained [103]. Additionally, contaminations can damage the reputation of forensic laboratories and waste resources and time through the creation of incorrect investigative leads, especially if they are not detected promptly [102, 103]. For these reasons, it is essential to minimise the risk of contamination as much as possible.

To minimise the risk of contamination within forensic laboratories, factors involved in the occurrence of these events need to be understood. This understanding can be gained through research that relates to exhibit contamination and DNA transfer within forensic laboratories. Once an understanding of the mechanisms involved in inadvertent DNA transfer exists, best practice contamination minimisation procedures can be identified and implemented to assist with minimising the risk.

1.6.1.1 Appropriate Contamination Minimisation Training

By ensuring that all staff who may be involved in the handling of exhibits are aware of the risk of contaminations and are correctly trained, these events can be minimised. While the risk of contaminations is generally well understood by forensic laboratories, police officers may not have the same level of understanding, due to the differences in training regarding the handling of evidence. Victoria Police Forensic Services Department (VPFSD) are one forensic unit that have reviewed the training offered to police officers, to ensure that they are receiving adequate contamination minimisation training. Within this organisation there was very limited police officer training identified, which was specific to contamination minimisation [106]. An e-learning package which details the correct and incorrect handling of exhibits, and indicates how easily contamination can occur, was provided to all operational police officers, who were then required to demonstrate their understanding of the material [106]. Forensic laboratory staff training was also reviewed, and altered, to ensure that all workers understood the importance of contamination minimisation [106]. As this is a recent process, there is currently no information reported which indicates whether the training package has decreased the number of police and forensic staff contaminations.

1.6.1.2 Forensic Laboratory Cleaning Regimes

To minimise the background level of DNA present in a laboratory, regular cleaning of surfaces and equipment is recommended. Previous studies have investigated the efficiency of a number of methods to remove DNA from equipment and laboratory surfaces [107, 108]. In one study it was determined that compared to six other cleaning methods, which involved various concentrations of water, ethanol and sodium hypochlorite, the most effective method involved the use of 0.9-1.8 % hypochlorite solution [108]. Another study found that cleaning with a 1 % hypochlorite solution, followed by 70 % ethanol was most efficient at removing DNA and other biological fluids from smooth and porous surfaces, however the combination of chemicals produced hazardous gases above recommended limits of exposure [107]. Due to this, a method which involved cleaning with a hypochlorite solution, followed by distilled water was trialled and although slightly less efficient, it was observed to be safer [107]. This cleaning method was introduced into the VPFSD laboratory and environmental DNA monitoring was performed to ensure that these cleaning methods were efficient. It was determined that after cleaning, trace amounts of DNA remained on surfaces at a level which were unlikely to be detectable if transferred [107]. Many laboratories have their own established environmental DNA monitoring programs to ensure that cleaning methods are effective and identify surfaces or items which pose a high risk of contamination [100, 109].

A number of regularly touched surfaces within DNA examination laboratories and at crime scene departments in Norway, have also been sampled to monitor the background levels of contaminating DNA [104]. Areas sampled within the laboratories were categorised as either high, medium or low risk areas, depending on the frequency of contact during examination [104]. It was

observed that medium risk surfaces, such as a camera tripod, DNA sampling equipment case, crime light and glove box, contained the most DNA. Significant quantities of DNA were also recovered from surfaces considered as high and low risk [104].

Studies involving the sampling of workspaces within other forensic laboratories, such as the VPSFD, determined that levels of DNA which were found on laboratory surfaces, and previously undetectable, can now be identified with the use of more sensitive technologies [105].

Another study investigated the levels of DNA which are present within SAPOL laboratories, where exhibit examinations may occasionally take place [21]. It was determined that significant levels of background DNA were found within SAPOL laboratories [21]. This information is crucial when considering the movement and processing of exhibits within SAPOL laboratories that occasionally occurs in South Australia.

While several studies investigate the transfer of DNA to items and workspaces in operational forensic laboratories [65, 94, 105, 107, 110-115], fewer studies have researched the persistence and transfer of cellular material in forensic workspaces outside of designated DNA-free examination areas. In a study conducted in 2016, surfaces and items across the biology floor of FSSA were sampled to measure the accumulating levels of DNA and determine its source [116]. It was observed that an individual's DNA was commonly found on items they touch frequently and within areas that they inhabit. However, the DNA from some individuals was also found in areas which they did not frequent [116]. In another study, which investigated the transfer of DNA within a forensic laboratory office workspace, similar results were obtained [117]. Individuals who regularly used an office area were often found to be the major contributor in DNA profiles generated from workspace samples [117]. In some instances, DNA could also be detected on items and surfaces from temporary office users. DNA which could not be attributed to temporary or frequent office users was also detected on some surfaces [117].

The results of these studies demonstrate the importance of also investigating the transfer of DNA in forensic laboratory areas, which are located outside of the designated DNA free examination laboratories. Currently there are limited studies which investigate the transfer of DNA in exhibit storage locations, or workspaces outside of the laboratory, where exhibits are handled.

1.6.1.3 Use of PPE

Individuals have been shown to deposit DNA through a variety of mechanisms, including direct contact, speaking, coughing and sneezing [59-61], therefore it is recommended that PPE such as gloves, a facemask and laboratory coat are worn within laboratories, especially where exhibits or samples are stored and processed, to minimise staff contaminations. The frequent changing of gloves in-between the handling of exhibits and laboratory equipment is also recommended [110, 114, 115, 118].

1.6.1.4 Laboratory Equipment Contamination Research

There are several studies which investigate the potential for items used within forensic laboratories to act as DNA transfer vectors. In numerous studies, gloves have been observed to efficiently transfer DNA between several different substrates [110-112, 114, 115, 118]. In one particular study, DNA from the examiner, other staff members and case associated persons of interest were observed within the profiles generated from an examiner's glove [110]. Other studies have shown that throughout the initial process of putting gloves on, DNA is added to the exterior surface [110, 111, 118]. DNA profiles containing varying numbers of alleles have also been generated from gloves which had not been used [111].

Laboratory equipment used during the examination of exhibits, such as forceps and scissors, have also been identified to be efficient DNA transfer vectors [94, 112, 114, 115]. DNA transfer was observed to occur between fabric, which had been touched once with forceps, or cut once with scissors, after they had contacted a substrate loaded with a trace amount of DNA [114]. In both heavy contact conditions (many contacts between the DNA loaded substrate and item) and light contact conditions (a single contact between the DNA loaded substrate and item) almost all alleles from the DNA donor were recovered from scissors [114]. In comparison, highly informative DNA profiles were only recovered from forceps in heavy contact conditions, however, DNA transfer was still observed to occur in light transfer conditions [114].

The potential for incidental DNA transfer to occur through equipment and solutions used during fingerprint analysis has also been identified [113, 119-121]. One study determined that varying amounts of DNA were able to be transferred between two surfaces through both fibreglass and squirrel hair fingerprint brushes [121]. The amount of DNA transferred, depended on the quantity of DNA deposited onto the initial substrate contacted. DNA quantities of up to 1 ng were recovered from unused squirrel hair fingerprint brushes, however no DNA was detected on the fibreglass brushes claimed to be DNA free [121].

Processes which involve submerging exhibits into solutions during fingerprint analysis, were also identified to present the risk of contamination [122]. Alleles that did not correspond to the fingermark donor were recovered from items after submersion [122]. Contaminating alleles were also recovered from blank sheets of paper, which were immersed into the solutions before and after the items containing fingermarks were submerged [122].

*1.6.1.5 E*xhibit Packaging Contamination Research

As PPE is generally not required to handle evidence bags prior to reaching the laboratory, it is assumed that the bag exterior accumulates DNA. However, there is limited research which explores the potential for evidence bags to act as DNA transfer vectors. One study demonstrated the ability for DNA to be transferred from an exhibit inside of a paper evidence bag, to other areas

of the interior surface of the bag [123]. It was also observed that DNA may be transferred from the initial deposition location on an exhibit, to other areas of the exhibit [123]. This is one of the only studies currently available that investigates the impact of packaging method on exhibits which contain biological materials [123].

In a previous study, preliminary research was conducted to determine whether DNA can be transferred from the exterior of an exhibit bag to the exhibit inside [104]. It was determined that even when strict contamination minimisation procedures were implemented, DNA could be transferred from the exterior of a bag to the exhibit inside during the examination process [104]. The contaminations in this study involved evidence bags that had the highest amounts of DNA detected on the exterior of the bag. This result suggests that the more DNA present on the outside of the bag, the higher the chance of incidental contamination [104]. Within this research, an insight into the amount of DNA accumulating on the exterior of exhibit bags at The Norwegian Institute of Public Health (NIPH) was also gained. Variable levels of DNA were identified on the exterior of casework evidence bags sampled [104].

Another study aimed to investigate the DNA load accumulating on the exterior of casework evidence bags throughout the handling and storage of exhibits [124]. It was observed that highly variable levels of DNA accumulate on the exterior of evidence bags throughout the handling and storage process [124]. It was also determined that during both the exhibit packaging and removal process, which occurs at the laboratory during the examination process, and at a crime scene, DNA can be transferred from the exhibit to the exterior of an evidence bag [124]. With the small sample size and number of replicates used within these experiments, this preliminary study highlights the need for further research within this area to be performed, in order to fully understand the potential risk of incidental exhibit contaminations through evidence bags [124].

Compared to studies which investigate the transfer of DNA to laboratory surfaces and equipment, there are limited studies that investigate the potential for evidence bags to act as DNA transfer vectors. While previous research indicates that DNA is present on the exterior of exhibit packages, there are currently limited studies available which provide an insight into the ability of this DNA to be transferred from exhibit packages to other surfaces, or between packages. There are also no studies which indicate the potential for DNA to be transferred through the porous surface of paper evidence bags.

1.6.2 Contamination Detection

Even with strict contamination minimisation procedures implemented within laboratories, contamination still occurs, therefore the ability to detect these events is vital. Many forensic laboratories utilise a laboratory elimination database to detect staff contamination. These databases contain the DNA profiles from forensic staff, police officers and other individuals who may attend crime scenes or are involved in processing of evidence. By comparison of the staff

database to DNA profiles produced from all samples processed within a laboratory, incidental contaminations from individuals who are on the database can be identified. Through studies that have identified instances of previously undetected staff contamination, the importance of all members involved in the handling or processing of evidence being on a laboratory elimination database has been made clear [104, 125, 126]. The necessity of staff elimination databases has also been reinforced in studies which detail the transfer of DNA within forensic laboratories [104, 124, 125].

While measures exist to detect contamination by forensic staff, sample to sample contamination is more likely to go undetected due to the difficulty to detect it and should be avoided at all costs.

1.7 Thesis Aims

The overarching aim of this research was to improve the understanding of the potential for exhibit contamination to occur during various stages of exhibit processing to allow forensic laboratories to assess whether existing procedures are still adequate given highly sensitive modern DNA profiling technologies. To achieve this aim, a combination of peer-reviewed manuscripts and unpublished research have been compiled into the following chapters:

Chapter 1: Introduction

This chapter outlines the background information relevant to the body chapters of the thesis.

Chapter 2: DNA Accumulation on Evidence Bags

This chapter aimed to further explore the potential for evidence bags to act as DNA transfer vectors by examining the DNA quantity and composition on the exterior of evidence bags before and after the exhibit examination process. This chapter consists of one manuscript.

Chapter 3: DNA within Forensic Workspaces

The aim of this chapter was to provide a better understanding of the level of DNA transfer that occurs between exhibit packaging and forensic workspaces and investigate the levels and sources of DNA that accumulate in forensic workspaces where exhibits are handled and stored. This chapter consists of one manuscript.

Chapter 4: DNA Transfer Between and Through Evidence Bags

The aim of this chapter was to understand the mechanisms behind the accumulation of exhibit DNA and the potential for evidence bags to act as DNA transfer vectors. This chapter consists of one manuscript draft and one poster presentation.

Chapter 5: Conclusions and Impact

The aim of this chapter was to provide a summary of the work within this thesis and discussion regarding the importance and future impacts that this work has for operational forensic laboratories and the wider general forensic community.

Chapter 6: Appendices

This chapter includes a publication and a poster presentation which was a result of research conducted immediately prior to the candidature. The manuscript was prepared and published, and the poster was presented during the candidature. This work has been added for reasons of completeness of the work conducted during the candidature.

1.8 Concluding Remarks

With so many factors that influence the deposition and persistence of DNA, it is difficult to determine how DNA was deposited onto a surface based on the quantity of DNA recovered, or the quality of a profile produced. This highlights the importance of research, which investigates the transfer and persistence of DNA on workspaces and items within forensic laboratories.

With highly sensitive DNA technologies that allow previously undetectable quantities of DNA to be observed, the potential to detect contaminations has increased. As the sensitivity of DNA technologies continues to improve over time, this is likely to become even more prominent in the future. As contamination events can occur at any stage of the processing of evidence, items and processes, which may allow the incidental transfer of extraneous DNA to exhibits need to be identified. Further research is required to ensure that current contamination minimisation procedures are still effective given the highly sensitive profiling technologies.

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CHAPTER 2 : DNA ACCUMULATION ON EVIDENCE BAGS

2.1 Preface

There are limited studies which explore the levels of DNA accumulating on the exterior of evidence bags and no investigation into the sources of accumulating DNA. To better understand the potential exhibit contamination risk posed by evidence bags, the levels of DNA accumulating on the exterior of the bags needs to be determined. This chapter explores the level of DNA accumulating on the exterior surface of paper evidence bags, through routine handling during casework. The exterior of casework evidence bags was sampled before and after examination of the exhibit inside to determine how the DNA composition changes as a result of the exhibit examination process and also identify the origins of accumulating DNA. Results are reported within a manuscript which was accepted for publication in Forensic Science International: Genetics, 57:102652. https://doi.org/10.1016/j.fsigen.2021.102652

2.2 Statement of authorship

Author	Contribution (%)			Signature	Date
	Research Design	Data collection and analysis	Writing and editing		
Claire Mercer	**85**	**80**	**70**	**Claire Mercer**	**7/11/2023**
Julianne Henry	10	5	10	Julianne Henry	7/11/2023
Duncan Taylor	5	10	10	Duncan Taylor	7/11/2023
Adrian Linacre	5	5	10	Adrian Linacre	24/10/2023

2.3 Manuscript: What's on the bag? The DNA composition of evidence bags pre- and post-exhibit examination.

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2.3.1 Abstract

Current forensic DNA profiling kits and techniques enable the detection of trace amounts of DNA. With advancements in kit sensitivity, there is an increased probability of detecting DNA from contamination. Research into DNA transfer within operational forensic laboratories provides insight into the possible mechanisms that may lead to exhibit contamination. To gain a greater understanding of the potential for evidence bags to act as DNA transfer vectors, the level of DNA accumulating on the exterior of evidence bags during the exhibit examination process was investigated. The exterior of 60 evidence bags were tapelifted before and after the examination of the exhibit inside of the bag resulting in 120 DNA profiles. These DNA profiles were compared to DNA profiles of staff working within the building and samples taken from the exhibit inside the bag. Common DNA profile contributors from each sample were also identified through STRmix™ mixture to mixture analysis. The average DNA quantity and number of profile contributors was higher in samples taken from the bag before exhibit examination than after examination. Fifty six percent of all samples taken identified a match between DNA recovered from the evidence bag and at least one staff member. On 11 bags, a common contributor was identified between the exhibit in the bag and the exhibit package post-examination. In one instance a DNA profile, matching that of a donor, on the exhibit bag before examination was also detected on a sample taken from the exhibit, raising the possibility of outer bag-to-exhibit DNA contamination. This study demonstrates that operational forensic laboratories must consider exhibit packages as a potential source of DNA contamination and evaluate their exhibit handling and storage procedures accordingly.

2.3.2 Introduction

Transfer of DNA between two substrates through direct contact is a well-established concept [1-5]. Further transfer of this DNA through subsequent contacts with other substrates, referred to as indirect transfer, has also been demonstrated [3-6]. With DNA technologies becoming increasingly sensitive, the probability of detecting DNA amounts originating from higher order transfers increases. Hence, the risk of detecting DNA that has been transferred to exhibits during the forensic process, and is not related to an alleged offence, has also subsequently increased. Such contamination events can have severe consequences for the outcome of criminal investigations. If a contamination goes undetected, it can mislead investigations, which may result in wasted

resources and miscarriages of justice [7]. Additionally, contaminating DNA can create a mixed DNA profile or mask the offender's profile from a sample, which can decrease the evidential value of a match with a person of interest or result in the loss of information that could have been used to identify an individual [7, 8].

Accredited forensic laboratories have procedures in place to prevent person to sample contamination, which include the use of personal protective equipment (PPE) in areas where DNA exhibits and samples are processed and frequent cleaning of equipment and workspaces. Some laboratories also use environmental monitoring programs to assess the levels of background DNA in the laboratory environment to ensure that cleaning regimes are effective [9, 10]. It is also standard practice for laboratories to establish a register of staff DNA profiles to identify contamination by an operator during examination or sampling of an exhibit [11, 12]. While procedures exist to minimise and identify contamination events in forensic DNA laboratories, it is important to understand how or when DNA is transferred so that high-risk practices can be identified and modified.

Many studies have investigated the potential for exhibit contamination through inadvertent transfer of DNA from laboratory equipment and workspaces [3, 13-17]. Gloves and examination tools, such as forceps and scissors, have been identified to be efficient DNA transfer vectors, and therefore pose a high contamination risk [3, 13-16, 18-20]. In multiple studies, DNA from mock exhibits was observed on secondary substrates after contact with previously used scissors, forceps and gloves [14, 15]. Residual DNA could still be detected on these items after subsequent contacts with secondary surfaces [13]. DNA from examination staff and case-associated persons of interest has also previously been detected in samples taken from the gloves used during the examination of casework exhibits [19]. Mercer et al. [21] observed that significant levels of DNA could accumulate on the exterior surface of an exhibit bag through routine handling, movement or storage of an exhibit. This is unsurprising as packages are typically handled without PPE and come into contact with numerous surfaces which are not free from DNA. More concerning is the demonstration that DNA present on the external bag surface can be subsequently transferred to the exhibit inside the bag [17] and from the exhibit inside the bag to the external bag surface [21]. Fonneløp et al. [17] observed one instance where a full profile from an individual who handled the outside of an evidence bag was generated from a swab inside, even though it was handled far from the bag. Additional contamination events were observed, where full or partial profiles from the bag handler were detected on items, when the bag was handled above the exhibit [17]. Demonstration of indirect DNA transfer, with the exhibit bag as the intermediary vector, is of concern as it creates the potential for cross-contamination between exhibits leading to false inclusions. However, data regarding the risk that DNA transfer from exhibit bags pose to the integrity of DNA evidence are limited and further research is required. Research which identifies the composition and origin of

DNA on exhibit bags is also required to understand how it comes to be there and develop work practices which minimise it.

This study explored the potential for evidence bags to act as DNA transfer vectors by examining DNA quantity and composition on the exterior of evidence bags before and after the exhibit examination process. Additionally, DNA transfer between an exhibit and the exterior of its package, were identified by determining the source of accumulating DNA. Information about the origin of DNA accumulating on evidence bags, and the transfer mechanisms involved can be used to assess and improve current exhibit handling and storage procedures to minimise the potential for exhibit contamination.

2.3.3 Materials and methods

2.3.3.1 Exhibit bags

Sixty casework evidence bags from routine case examinations at Forensic Science SA (FSSA) were sampled, before and after the examination of the exhibit inside of the bag. Samples were taken before the exhibit was removed from the bag and directly after the exhibit was repackaged, and the bag was resealed. Evidence bags were received from different police local service areas (LSA) and contained different types of exhibits (Table 2.1). All bags were composed of brown paper, but their size and brand varied depending on the police LSA and size of the exhibit. The approximate size of brown paper bags sampled in this study was 30 x 40 cm. An example of one of the exhibit bags sampled in this study is shown in Supplementary Figure 2.7. Example of an exhibit bag tapelifted in this study, with the primary sampling area shaded in red. For the alternative sampling method, the shaded top half was tapelifted before exhibit examination and the unshaded bottom half was tapelifted after exhibit examination. Samples were collected in designated evidence recovery laboratories, by examiners wearing a face mask, laboratory gown, hair net and gloves.

Table 2.1. The type of exhibits inside each of the evidence bags sampled. The biological material sampled on each exhibit is indicated. Numbers that are underlined indicate exhibits which were sampled for trace DNA in addition to another biological material.

Exhibit type	Number of bags containing each exhibit type	Number of exhibits sampled for each biological material				
		Trace only	Blood	Saliva	Semen	Tissue
Jacket / Jumper	6	4	2			
Glove	10	10				
Headwear (beanie, cap, hat, bicycle helmet)	13	13				
Face mask	3	1	1	1		
Underwear	2	1			1	
Bag (plastic, cloth)	4	4				
Pillowcase	1		1			
Plastic bottle	1			1		
Sock	1	1				
Shoe (sand shoe, thong, sandal)	3	3				
Paper towel/ Tissue	1	1				1
Wooden frame	1	1				
Handkerchief	1	1				
Tools (Hammer, Crowbar)	2	1	1			
Heat sealing machine	1	1				
T-shirt	3	2		1		
Pants (trousers, jeans, leggings)	3	3				
Nightie	1	1				
Clear tape	1	1				
Mattress Cover	1	1				
TOTAL	60	50	5	3	1	1

2.3.3.2 Sampling of exhibit bags

2.3.3.2.1 Method 1 (primary sampling method):

As indicated in Figure 2.7, the top half (including front, back and sides) of the bag exterior, which included the evidence tape seal, was sampled on 50 bags, using DNA Tapelift Kits (Lovell Surgical Solutions, Melbourne, Australia) which were repeatedly pressed onto the bag's surface until the designated area was sampled, or adhesiveness of the tapelift was lost. The same area of the bag was tapelifted before and after exhibit examination.

2.3.3.2.2 Method 2 (alternative sampling method):

When sampling using the primary sampling method there was a risk that the sampling that occurred prior to exhibit examination was removing DNA, and this could lead to apparently lower amounts of DNA in the samples taken post examination. To determine if the amount of DNA

recovered from the bag post-examination was being reduced by the pre-examination sampling, a second approach was employed where the top half of the bag was tapelifted pre-examination and the bottom half post-examination. With the variation in exhibit packaging received there is not always a clearly defined 'top' and 'bottom' of the bag which is predominantly gripped during handling. The packaging of exhibits is not consistent and bags can be labelled and stored in any orientation. Bags are extensively handled and may be contacted in differing regions, depending on the orientation of the bag and the shape of exhibit inside. For this reason, it was assumed that the manner of contact between the designated top and bottom half of the bag did not differ. An additional 10 bags were sampled using the alternative sampling approach.

2.3.3.3 DNA analysis

DNA analyses were performed using in-house validated protocols at FSSA. DNA was extracted using the DNA IQ system (Promega, Madison, WI, USA) on a Microlab® AutoLys STAR Liquid Handling Platform (LHP) (Hamilton Company, Reno, NV, USA), with an elution volume of 60 µL. Samples were quantified using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific, Waltham, MA, USA), on an ABI PRISM® 7500 Sequence Detection System (Thermo Fisher Scientific). DNA was amplified using the GlobalFiler™ PCR amplification kit (Thermo Fisher Scientific) on a ProFlex™ Dual 96-Well PCR System (Thermo Fisher Scientific). Cycling conditions were as per manufacturer's recommendations for 29 cycles with either 400 pg of DNA or 15 µL DNA extract (where 400 pg DNA was not available). Amplified DNA fragments were electrophoresed on a 3500*xl* Genetic Analyser (Thermo Fisher Scientific).

2.3.3.4 DNA profile analysis and comparison

All 120 DNA profiles taken from the bags were analysed using GeneMapper® ID-X Software v1.6 (Thermo Fisher Scientific) with an allele analytical threshold (AT) of 50 relative fluorescence units (RFU). The minimum number of contributors required to reasonably explain each profile was determined, using a minimum allele count and taking into consideration peak heights. STRmix™ V2.7 was used to deconvolute DNA profiles into individual contributor profiles using in-house derived settings. Due to limitations in computing power, profiles containing more than four contributors (N=82) could not be analysed in STRmix™ and were re-analysed in GeneMapper® ID-X using an AT of 250 RFU, to capture the major profile contributors. This is not a practise carried out at FSSA in casework but was applied here to obtain the maximum amount of useful information. This is the same methodology employed in another study [9] and in practise is similar to the process of carrying out a top-down analysis [22, 23]. When the elevated AT was used, the number of contributors were assessed in the re-analysed profiles, and those which still contained more than four contributors were not analysed further (N=21).

Profiles generated from the evidence bags were compared to de-identified DNA profiles on the FSSA staff elimination database (N=1801). The staff elimination database contains DNA profiles of

all individuals who work at FSSA (including contractors, students and laboratory visitors), crime scene examiners and other individuals who may attend crime scenes or be involved in the handling of exhibits (police officers and doctors). Of the 1801 individuals on the database, 81 provided informed consent to be identified from their DNA profile within this study. For each comparison, a likelihood ratio (LR) was calculated using the opposing H1 and H2 propositions:

H1) the staff member and (N-1) unrelated individuals are the sources of DNA

H2) N unknown individuals, unrelated to the staff member are the sources of DNA

(where N is the number of profile contributors). An account for co-ancestry was not applied to the LR calculations and the point estimate value was used. The Australian Caucasian allele frequency data was used for LR calculations [24]. A minimum LR cut-off of 10,000 was used to minimise adventitious matches, as per standard FSSA protocol. To better resolve the genotypes of unknown individuals, profiles with staff matches were re-analysed in STRmix™ with the matched individual assumed as a contributor [25].

The mixture to mixture comparison function of STRmix™ was used to compare DNA profiles from bag samples taken before and after exhibit examination. For each comparison, a LR was produced using the propositions:

H1) the two mixtures share a common donor or

H2) there are no common contributors within the two mixtures.

Again, a minimum LR threshold of 10,000 was also used in mixture-to-mixture comparisons. There were 18 exhibit bags where a mixture comparison could not be performed, due to the complexity of profiles generated from the bag samples.

Mixture-to-mixture analysis was also used to compare the DNA profiles in both samples taken from the bag and the 67 profiles from samples taken from the exhibit inside of the bag. Exhibit profiles that were previously determined to contain more than four contributors were re-analysed in GeneMapper® ID-X using an allele detection limit of 250 RFU (N= 17). Sixteen of these re-analysed exhibit profiles were able to be further used, one could not be due to its complexity.

2.3.3.5 Statistical analysis

Mann-Whitney U Tests were used to compare the DNA quantities and minimum number of profile contributors in samples taken before and after exhibit examination. The quantities of DNA and number of profile contributors recovered from the bags using the ‘primary’ and ‘alternative’ sampling approach were also compared using a Mann-Whitney U Test.

There were not enough bags which contained exhibits with different biological materials to determine whether there was any correlation between the quantity of DNA recovered from the exterior of the bag and each type of biological material. Instead, the DNA quantity and number of contributors for bags containing exhibits with trace DNA (typically low amounts of DNA) were compared to those containing exhibits with non-trace biological material (typically high amounts of DNA) using a Mann-Whitney U Test. Regression analysis was also performed, to determine whether there was a correlation between exhibit storage time and the DNA quantity or number of profile contributors in samples taken from the bag before exhibit examination. In all statistical tests, a significance level of $p < 0.05$ was used.

2.3.4 Results

2.3.4.1 DNA yield and profile contributors

Casework exhibit bags were sampled to investigate the composition of DNA on the exterior of evidence bags and explore how it may be altered during the exhibit examination process. DNA profiles were produced from all 120 tapelifts taken within this study. Minimum numbers of contributors to all profiles were assessed and these values are displayed in Figure 2.1, along with the DNA quantities.

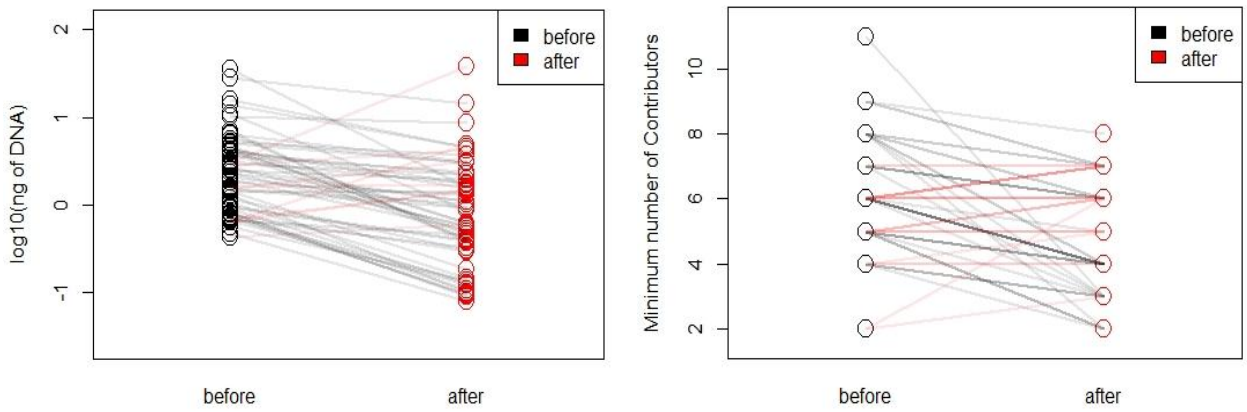

Figure 2.1. The log10 DNA quantities (ng) recovered from evidence bags before and after exhibit examination are displayed on the left side of the figure. The number of contributors to profiles generated from bag samples taken before and after exhibit examination are displayed on the right side of the figure. Numbers of profile contributors were assessed using a peak detection threshold of 50 RFU. Red lines represent instances where the DNA quantity or number of profile contributors in the sample taken after exhibit examination was higher than the sample taken before examination. Grey lines represent a decrease or no change in the DNA quantity or number of contributors between the samples taken from the bag before and after examination.

Highly variable quantities of DNA were recovered from evidence bags. DNA quantities between 0.4-36 ng were observed pre- examination, while values between 0.07-37 ng were detected post-examination. The sampling method did not have a significant effect on the magnitude of

differences in DNA amount seen before and after examination (p=0.71). For each sampling method, the difference in numbers of profile contributors observed pre- and post- exhibit examination were also compared, and no statistically significant difference was identified (p=0.26). As there was no difference in the DNA quantities or number of profile contributors between sampling methods, results from all tapelifts have been combined in Figures 2.1, 2.2, 2.3, 2.5 and 2. 6, Table 2.2 and further analysis.

On 44 of the 50 bags tapelifted using the primary sampling method, there was a higher DNA quantity before exhibit examination, compared to after examination. For this sampling approach, the average quantity of DNA recovered from the bag before exhibit examination was significantly higher than samples taken post examination (p=0.00016). On 34 of the 50 exhibit bags tapelifted using the primary sampling method, there was a higher number of contributors observed before exhibit examination compared to after examination. On eight bags, the same number of contributors were observed both before and after exhibit examination. On the remaining eight bags, a higher number of contributors were observed after exhibit examination, compared to before examination. For samples taken using the primary sampling method, the average number of contributors observed on bags before exhibit examination was significantly higher than after examination (p=0.0022).

On eight of the ten bags sampled using the alternative sampling method, a higher DNA quantity was observed before exhibit examination, compared to after examination. This decrease in DNA was also observed in the primary sampling approach but was expected to be a result of the initial tapelift removing DNA from the sampled area. As this same trend was observed within the alternative sampling approach, it is apparent that other factors may also have influenced the result. The average quantity of DNA recovered from the bag before exhibit examination was significantly higher than samples taken post examination (p=0.038) within the alternative sampling approach. Due to the smaller sample size within the alternative method, it was expected that p values may be less significant, compared to the primary method. Within this method there was no significant difference in the number of contributors on bags before and after examination (p=0.26). On four bags, a higher number of contributors were observed pre- examination, compared to post-examination. The opposite trend was observed on three exhibit bags, and on the remaining three bags, the same minimum number of contributors were observed both before and after examination.

Bags containing exhibits with non-trace materials (blood, semen, saliva, tissue) did not have significantly higher amounts of DNA recovered from the outer bag compared to exhibits with trace DNA. This was observed both before (p=0.61) and after (p=0.46) exhibit examination. There was no correlation between pre-examination exhibit storage time (displayed in Figure 2.4 and Supplementary material Figure 2.10) and the DNA quantity (p=0.72) or number of contributors (p=0.48) on the bag before examination.

2.3.4.2 Staff database and exhibit chain of custody comparisons

To determine the source of DNA accumulating on the exterior of evidence bags, profiles which were deemed to be analysable with STRmix™ were compared to DNA profiles on the FSSA elimination database. This allowed us to further investigate exhibit bags as a potential vector for DNA transfer and explore the general concepts of DNA transfer and persistence. The results from staff database comparisons are displayed in Figure 2.2.

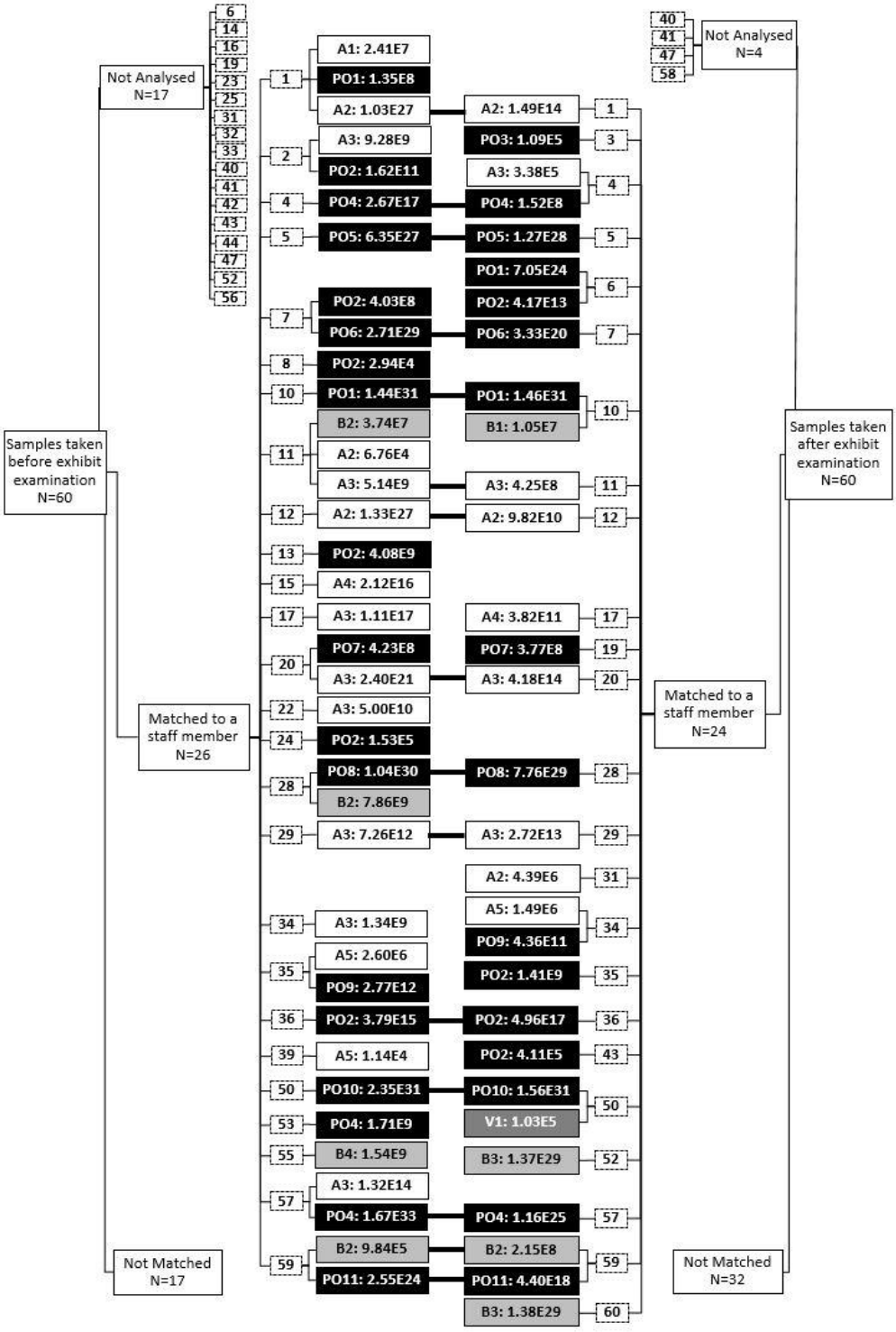

Figure 2.2. Samples taken from bags before exhibit examination are displayed on the left side of the figure, while samples taken after exhibit examination are displayed on the right side. On both sides,

samples have been divided in three categories to indicate the samples which were not analysed due to profile complexity, those which did not generate any matches to the staff database, and those that yielded a staff match with an LR > 10,000. The values in boxes with a dashed border represent the exhibit bag number. Individuals matched to a profile have been indicated using the following letters and shades for each workgroup: A=Admin (white box with black text), B=Biology (light grey box with black text), PO=Police (black box with white text) and V=Visitor (dark grey box with white text). Each individual has been assigned a number to differentiate multiple individuals within the same workgroup. The corresponding LR for each inclusion is also shown after the individual. Connecting lines have been used to indicate staff DNA that was on the bag before exhibit examination and persisted on the bag after examination. Bags 1-50 were sampled using the primary sampling method and bags 51-60 were sampled using the alternative sampling method.

Of the 120 DNA profiles produced, 41% generated an inclusionary LR to at least one individual on the elimination database, 41% did not generate any matches and 18% were too complex for analysis. At least one staff match was generated to 43% of samples taken before exhibit examination and 40% of the samples taken after exhibit examination. The breakdown of matches to individuals in specific workgroups is shown in Figure 2.3.

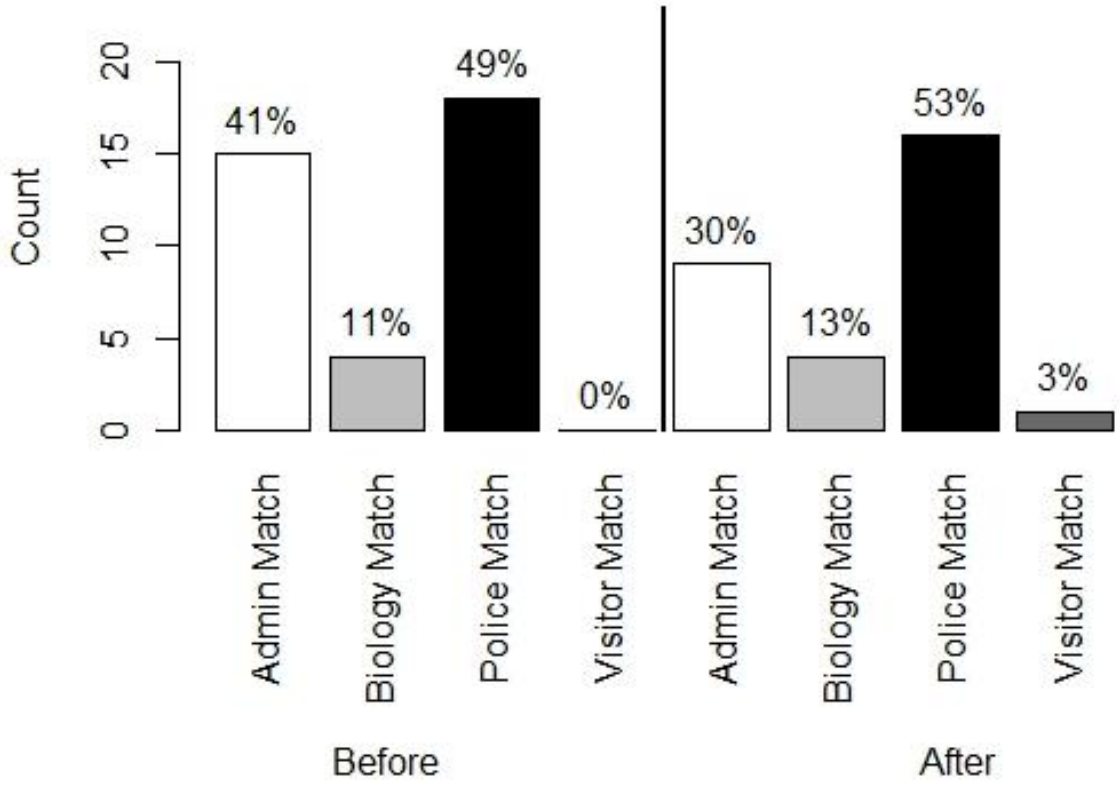

Figure 2.3. Breakdown of the 41% of comparisons of the 120 profiles generated from bag samples, showing proportions of individuals from each FSSA department that were matched to the samples taken from bags before and after exhibit examination.

By comparing the FSSA chain of custody records to staff matches, mechanisms whereby an individual's DNA came to be on the exhibit bag were investigated. The chain of custody for all exhibit bags is depicted in Figure 2.10 to Figure 2.12. For reasons of brevity, the chain of custody for four of the 60 exhibit bags sampled within this study has been depicted in Figure 2.4.

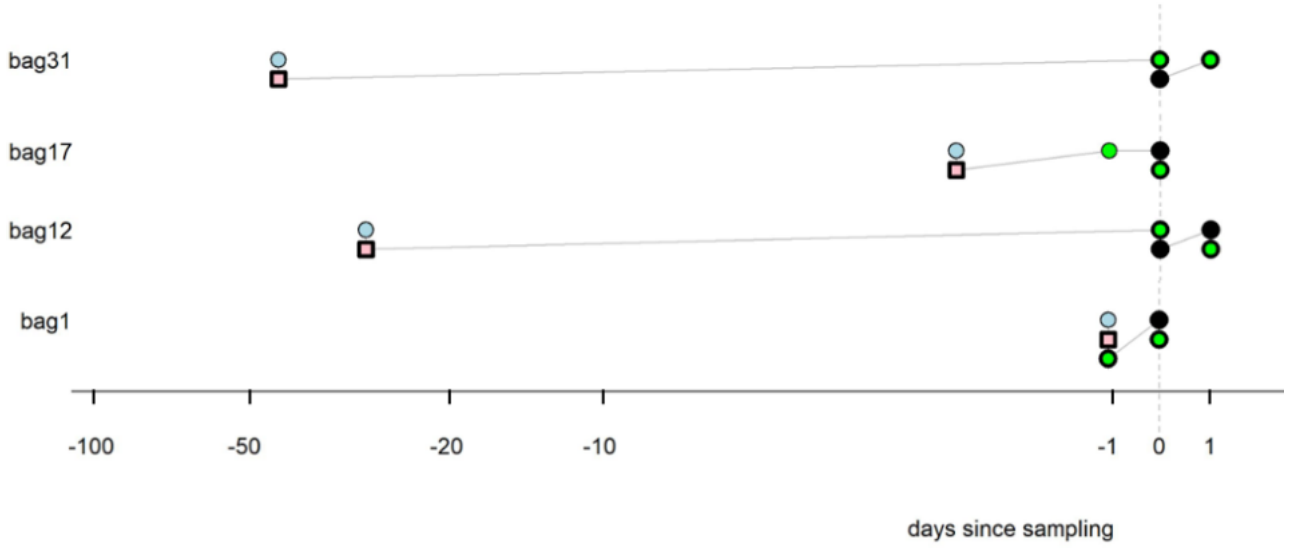

Figure 2.4. Chain of custody for four exhibit bags sampled within this study. The bag number is shown on the vertical axis and the horizontal axis represents the number of days relative to the first sample being taken from the bag. Individuals who handled the bags have been represented with a symbol. Circles indicate individuals who were identified as contacting the bag in the chain of custody but were not detected on the bag. Squares indicate individuals who were identified as contacting the bag in the chain of custody and detected on the bag either before or after. Symbols with bold borders represent individuals who provided consent to have their DNA identified. The colour of the symbols represents individuals from different departments (blue= police, red= admin, green= biology). Black circles indicate the bag sampling points. For bags where multiple movements occurred on the same day, symbols are vertically stacked with the first movement at the top of the stack.

Twenty-one individuals generated an LR which favoured their inclusion to at least one bag sample. Six of these individuals provided informed consent to have their DNA profile identified. For individuals who were present on the exhibit chain of custody and provided consent to have their DNA identified, a summary of the bags handled by the individual and the bags on which their DNA was detected, are shown in Table 2.2.

Table 2.2. Shaded bag numbers represent samples which were matched to the individual. Black shading represents a match between the individual and both samples taken from the bag. Light grey shading indicates a match between the sample taken from the bag before examination. Dark grey shading represents a match between the individual and the sample taken after examination.

STAFF REFERENCE	BAGS DIRECTLY HANDLED BY THE INDIVIDUAL
B1	4 **10** 20 22 49
B2	1 2 3 4 5 6 7 8 9 10 **11** 12 13 14 15 16 17 18 19 21 22 23 24 26 27 **28** 29 30 33 38 48 51 52 53 54 55 56 57 58 **59** 60
B3	25 30 40 50 51 **52** **60**
B4	6 8 14 23 25 31 32 33 38 41 45 47 48 49 50 54 **55** 57 58
B5	35 36 37 39 42 43 44 46
A2	**1** **12** **31**
A4	**15** 16 **17** 19 30 33 33 55 58 59
A6	50 60

Most commonly, individuals who yielded the largest LRs had not contacted the bag most recently. Some individuals who were known to handle exhibit bags did not deposit their DNA at all or in high enough quantities to produce inclusionary LRs. Some individuals were detected on exhibit bags more frequently than others. For example, an inclusionary LR was generated between 12 samples and individual A3, which was the highest number of matches observed to a single individual.

Of the 17 samples that were matched to an individual who provided consent to be identified, 16 were taken from a bag that the individual directly handled. There was one instance where an individual was not listed on the chain of custody for an exhibit, but their DNA was detected on the bag. This instance was a match between the sample taken from bag 11 before exhibit examination and individual A2.

With the chain of custody records, some inferences about possible DNA donors could also be made for unknown staff members who yielded an inclusionary LR. There was one instance where DNA from an individual who did not consent to identified, but is on the laboratory elimination database, was matched to a bag that they did not handle according to the chain of custody. This individual was a laboratory visitor, who was matched to the sample taken from bag 50 after exhibit examination. Instances where DNA from an individual was detected on a bag which they had not handled suggests that in-direct DNA transfer mechanisms are also responsible for some of the DNA accumulating on exhibit bags. Common DNA profiles of unknown individuals (i.e., individuals who were on the elimination database but had not given consent for their identity to be known) were also detected on multiple bags. While the chain of custody provides some insight into possible sources of unknown staff DNA, it could not be determined with certainty whether the other 15 individuals who generated an LR directly contacted the bags, as their DNA could not be

identified. This made it difficult to make inferences about the most likely explanation for the detection of these individual's DNA within a sample.

2.3.4.3 Mixture to mixture comparisons between samples taken from the same bag

To further investigate the possible origin of DNA, which was detected on bags, but could not be attributed to staff members whose DNA is on the laboratory elimination database, a comparison was performed between the contributors to profiles generated from samples taken pre- and post-exhibit examination, and to the DNA profiles generated from the exhibit inside the bag. The bags where a common non-staff contributor was identified between samples taken before and after exhibit examination are displayed in Figure 2.5.

No comparison between samples taken before and after examination was performed for 18 bags, due to the complexity of the resultant profiles. Of the remaining bags, 18 contained at least one common non-staff contributor between the samples taken before and after exhibit examination. Pre- and post-examination samples taken from seven of these bags did not yield any LRs to staff members or have a common contributor identified within the profile generated from the exhibit.

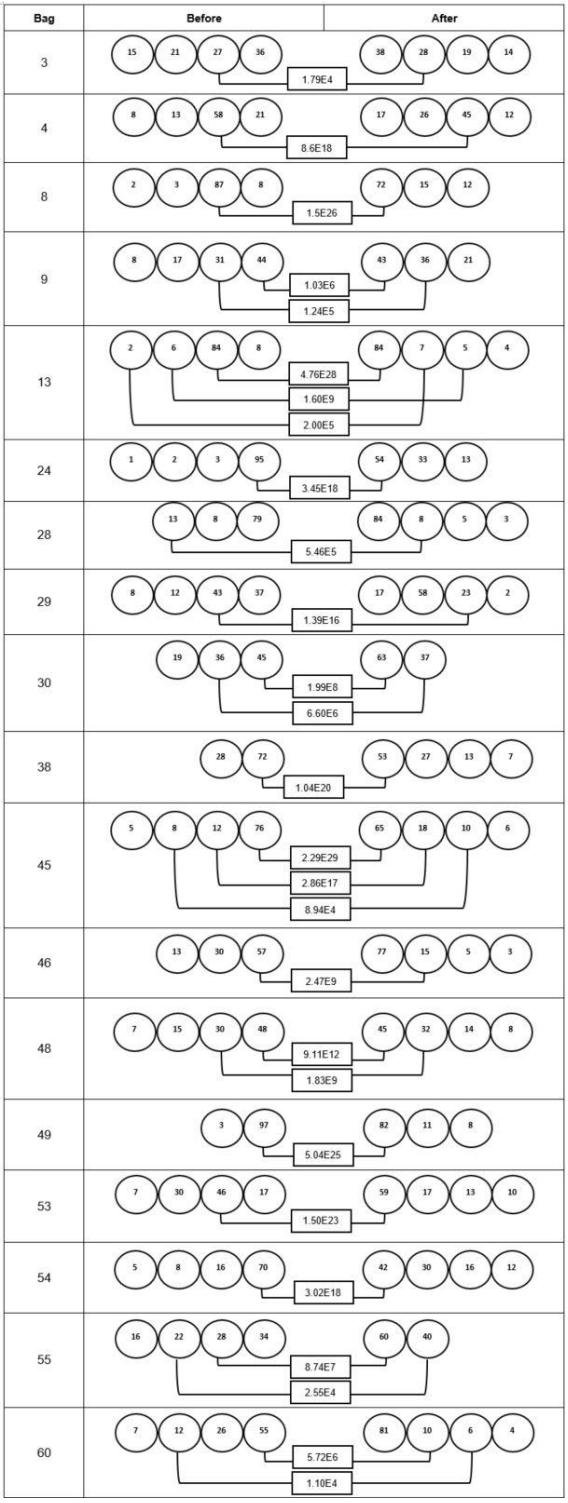

Figure 2.5. Mixture to mixture analysis results, where the number of contributors to profiles generated from samples taken from the bag pre-examination are displayed on the left and post- examination are displayed on the right. Circles have been used to indicate profile contributors and the contributor mixture proportion (rounded to the nearest whole number) has been written inside of each circle. Common contributors between each of the profiles are indicated using a connecting line with the corresponding LR for the inclusion in the attached box. Common contributors who could be accounted for through a staff member being matched to both samples from pre- and post- exhibit examination have not been displayed.

2.3.4.4 Mixture to mixture comparisons between bag samples and exhibit profiles

Diagrams which map each distinguishable DNA transfer or persistence event for bags where a common contributor was identified between a sample taken from the bag and the exhibit inside of the bag are displayed in Figure 2.13-Figure 2.24. For reasons of brevity, a summary diagram that contains the total number of staff matches observed, common contributors between samples, and common profile contributors identified between bag and exhibit samples are displayed in Figure 2.6.

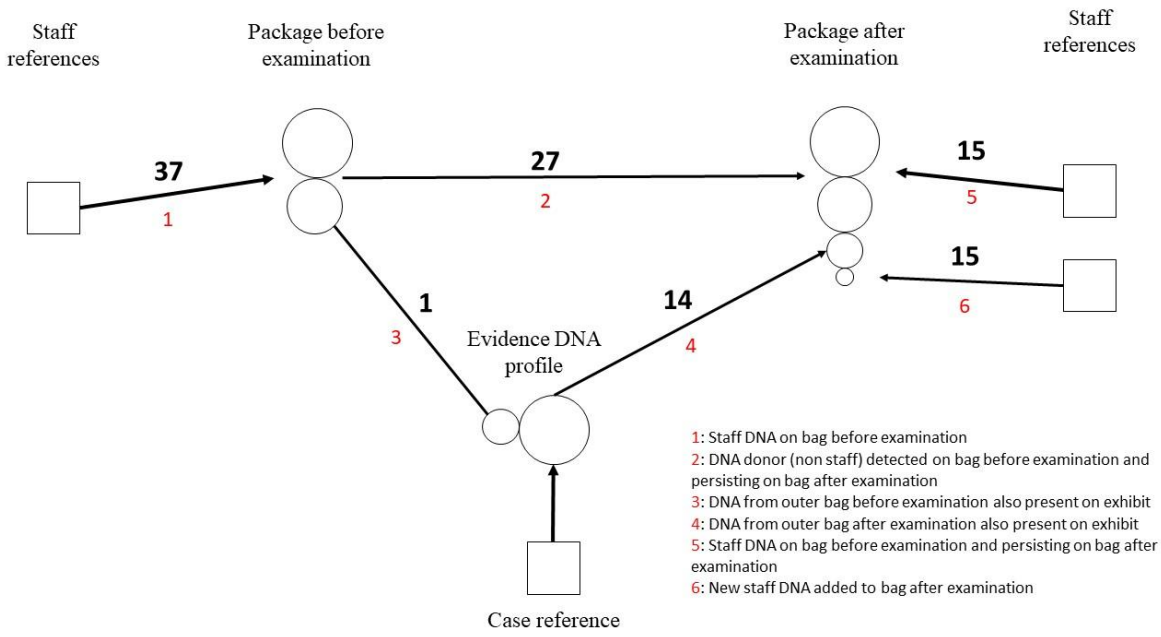

Figure 2.6. Summary DNA transfer diagram that indicates the DNA transfers observed within this study. Circles represent a mixture where the number of circles represent the number of contributors to DNA profiles generated from the bag before and after exhibit examination and from the exhibit inside of the bag. The square boxes represent staff references (as per Figure 2.2 and Table 2.2.) or case reference profiles. Arrows are used to indicate common contributors between profiles with the direction of the DNA transfer indicated (if known). There is no arrowhead for arrow 3, as the direction of transfer is unknown. Numbers displayed in red indicate the type of each observed event, as described by the text in the bottom right corner of the figure and bold black numbers indicate the number of instances of each event observed.

Due to a low DNA quantity being detected within samples, a profile was not generated from nine of the exhibits inside of the bags sampled. With the absence of these exhibit profiles and the portion of profiles generated from the bags that were too complex for analysis, 84 of the 120 bag samples were compared to the exhibit inside of the bag (36/60 samples before and 48/60 samples after).

There was at least one common contributor observed between the profile generated from the exhibit and a sample taken from the outside of its packaging for 12 bags. In eight of the 15

instances where a common donor was observed between the exhibit and the bag sample, the donor was the major contributor to both profiles.

Only one of the 36 samples taken from an exhibit bag before examination was identified to have a common donor with the sample taken from the exhibit inside of the bag. As the bag was sampled before the exhibit was removed from its package, this DNA could not have been transferred to the bag because of the examination process. This result raises the question of whether the DNA was transferred from the exterior of the evidence bag to the exhibit inside of the bag. The same donor was also identified in the sample taken from the exhibit bag after examination. Through comparison to the staff database, the common donor was determined to be a police employee. In this case, it cannot be determined whether DNA transfer occurred between the exhibit and the bag, or whether the DNA from this donor was transferred to the exhibit and the bag separately through unrelated transfer events.

At least one common donor was only observed between the exhibit and the sample taken from the bag after examination on the remaining 11 bags. For two of these bags, it could not be determined whether the DNA was also on the bag before examination, as the samples yielded a profile that was too complex for analysis. Since no common donors were identified between the exhibit and pre-examination samples from the remainder of bags, these results indicate that DNA from the exhibit was transferred to the exterior of these bags as a result of the examination process. The alternative is that the DNA was present on the outer surface of the bag initially, not detected during the initial sampling, then transferred to the exhibit during examination. Detection of exhibit DNA in post- examination bag samples raises the question of what mechanisms could be responsible for the transfer of DNA from an exhibit to its exterior package and whether this DNA is being further transferred to workspaces and other items within the laboratory.

2.3.5 Discussion

Experiments that investigate the accumulation of DNA on evidence bags, and the potential for exhibit packaging to act as DNA transfer vectors, provide valuable insight into the possible risk of exhibit contamination. The consideration of this information is important to assess and improve current exhibit handling and storage procedures.

There are many variations in the size and composition of exhibit packaging used, and while the movement and handling histories of all exhibits differs, this study provides insight into the levels of DNA accumulating on evidence bags. This study demonstrates that DNA can be transferred to evidence bags, via direct and in-direct mechanisms, throughout the various stages of exhibit handling, which occurs during criminal investigations. As contact between substrates provides the opportunity for bidirectional DNA transfer to occur [26, 27], it was expected that each exhibit bag would have a different DNA composition because of its unique movement history. This explains the

variation in DNA quantities and the number of profile contributors observed between bag samples and the results are consistent with findings from other studies [17, 21].

Most frequently, there was a decrease in DNA quantity and the number of contributors between the samples taken from the bag before and after examination. Between sampling points, bags were handled in designated 'DNA-free' laboratories by examiners wearing PPE. Gloves are known to be efficient DNA transfer vectors [15, 16, 18-20], therefore it is possible that DNA was removed from the bags through contact with the examiner's gloves while unpacking the exhibit. It is also possible that DNA was transferred from the bag to the surface or workspace where it was stored during exhibit examination, which reinforces the importance of cleaning laboratory surfaces. These concepts could be tested by sampling the gloves worn by examiners after handling evidence bags and the surfaces where exhibit packaging is stored during the examination of the exhibit. The ability for DNA to accumulate on the exterior of gloves reinforces the importance of frequently changing gloves throughout the examination process and particularly after contact with items which are not 'DNA-free'. Changing gloves immediately before and after removing exhibits from their package may reduce the risk of DNA being transferred between the exterior of the bag and the exhibit.

For the remaining few samples, there was an increase in DNA quantity and numbers of profile contributors between the samples taken before and after examination. For one of these instances, DNA transferred from the exhibit to the bag could explain this trend. In the other instances, DNA that was transferred into the sampling area via the examiner's gloves, from an area of the bag that was not sampled, may explain this result. It is also possible that DNA was added to the bag, from a surface where it was stored during exhibit examination.

As PPE is only required for handling exhibit packaging during the laboratory examination stage in our laboratory, it was expected that police and certain forensic employees may be a source of accumulating DNA. Previous studies show that DNA from individuals who have directly handled an exhibit bag without PPE can be detected on the bag's exterior [17]. Comparison of the exhibit chain of custody with staff inclusions provided some indication of the mechanisms, which may have resulted in DNA from a donor being detected on the bags. However, the exhibit chain of custody is not always a representation of all individuals who have contacted an exhibit bag, as entries are not present for instances where an individual has handled the exterior of an exhibit bag but not taken custody of it. One example of a situation where this may occur is when an individual contacts an exhibit bag while searching for another exhibit which is stored on the same shelf. The unknown occurrence of contact between exhibit bags and other substrates, such as shelves and workspaces, also makes it difficult to assess the exact origin or transfer mechanism of accumulating DNA.

LRs that favoured the inclusion of a staff member generally corresponded with the individual's regular work duties. All individuals from Biology who were matched to a sample, collected the

exhibit for examination and therefore handled the bag without PPE prior to sampling. Although a Biology employee handled all bags prior to examination, there were fewer inclusions to members of this workgroup, compared to Administration and Police. This could be due to a shorter contact time with the bags, as a basket is commonly used to move items from the storeroom to the laboratory and then PPE is used from that point onwards. The frequent handwashing by individuals from the Biology workgroup, as part of their normal work practices, may mean that they have less 'self' DNA on their hands compared to other individuals. It is unknown whether frequent glove use may also influence the amount of DNA deposited by individuals from Biology. In comparison, Police and FSSA Administration employees extensively handle exhibit bags during bag labelling, recording of item details and throughout the process of transporting items between storage areas.

As the departments of all individuals who matched to a sample were known, inclusions to unknown individuals could be accounted for with the assumption that an individual will deposit DNA within areas they frequent [9, 28], which can be transferred to other substrates through vectors [3, 5, 6, 29-32]. All unknown individuals, except for the laboratory visitor, are either police employees or work within the FSSA Administration department. These matches therefore correspond with frequent evidence bag handling, which is involved in the regular work duties of individuals from these workgroups. As it is not routine for a laboratory visitor to handle an evidence bag, it is expected that DNA from the individual was most likely detected on the bag as the result of an indirect transfer mechanism. One explanation for this inclusion could be that DNA was deposited onto a laboratory surface, and then transferred to the bag through contact with that surface.

Instances where individuals who yielded the largest LRs but had not contacted the bag most recently indicate that there may be variation in the amounts of DNA being deposited by different individuals. Some individuals were known to handle a bag but either did not deposit any DNA or enough DNA to generate an inclusion to those samples. This result suggests that some individuals may be more prone to depositing larger amounts of DNA than others and supports the concept of 'shedder status' [2, 33-37]. This concept was further supported by the result that some individuals generated inclusions to samples more frequently than others. However, it is possible that these individuals were inadvertently involved in the handling of more of the bags sampled, and therefore had more of an opportunity to directly deposit their DNA onto the bag than others.

Much of the DNA detected did not generate an inclusion to any individuals on the staff database or contain a common donor with the exhibit. Possible sources of the unknown DNA are 'non-self' DNA [36] that was transferred from individuals who have handled the exhibit bags or police employees who are not on the laboratory elimination database. As exhibit bags are not 'DNA free' before use, it is possible that DNA from individuals who are involved in the packaging or production of the bags is also present. These data demonstrate the importance of all individuals who are involved in the handling of exhibit bags, or work within workspaces where exhibits are handled,

being included in a laboratory elimination database. This would allow the detection of any staff contaminations, which may occur because of DNA transferred from the bag to the exhibit.

There were instances where DNA from the exhibit inside of the bag was detected on the exterior of the bag after exhibit examination. The mechanism that facilitated this transfer is unknown, but one possible explanation is that the exhibit very briefly contacted the exterior of the bag during the re-packaging process. Another possible explanation is that DNA from the exhibit is transferred onto the examiner's gloves, as in [19], and then onto the bag exterior, during repackaging. It is also possible that DNA containing material is dislodged from the exhibit and falls onto the exterior of the bag while repackaging or removing the item. This result emphasizes the opportunity for evidence bags to act as transfer vectors that facilitate the transfer of DNA from an exhibit to other exhibit bags and workspaces. Additionally, this result raises the question of whether exhibit DNA can accumulate within forensic workspaces, if transferred to the bag exterior during examination. Precautions should be taken to prevent DNA from the exhibit being transferred to the exterior of its packaging, as this creates the potential for DNA from case related persons of interest to be transferred to other non-related exhibits via exhibit packaging. To prevent the accumulation of exhibit DNA on the exterior of evidence bags, examiners should change gloves before repacking an exhibit and minimise contact with the outside of the bag. As there is limited research which investigates the transfer of DNA between exhibit bags and other substrates, further research is required to better understand this risk.

A previous study demonstrated the ability for DNA to be transferred from the exterior of the exhibit bag to the exhibit itself [17]. In this study, DNA transfer from the bag to the exhibit was observed in instances where the exhibit contained high quantities of DNA, which implies that the higher the amount of DNA on the bag exterior, the higher the risk there is of exhibit contamination [17]. While the risk of transfer from a bag to other surfaces is unknown, these findings reinforce the risk of bringing evidence bags, which are observed to accumulate DNA, into 'DNA-free' examination laboratories.

The extensive movement and handling of exhibits, both external and internal to an operational forensic laboratory, makes it difficult and likely unrealistic to implement procedures which result in exhibit bags being free of contaminating DNA before they enter an examination laboratory. Whilst introducing a requirement for exhibit bags to be handled by a person wearing PPE (or at least gloves) may reduce DNA transfer, this would be easier to implement in an operational environment where these practices are commonplace, compared to a crime scene or police station, where compliance would be often impractical. Frequent cleaning of areas where exhibits are handled and stored may also reduce the amount of DNA accumulating.

While preventing contact between the bags of different exhibits during storage and transport may reduce the risk of contamination from direct contact between evidence bags, the required space to transport and store exhibits in this manner may also be unrealistic and impractical.

‘Double bagging’ exhibits and removing the outermost layer once the item reaches the laboratory may reduce the amount of contaminating DNA brought into examination laboratories, however regardless of the exhibit packaging method used, at some point there must be an outermost surface that is considered ‘unclean’ as it is free to accumulate DNA from its surroundings in an uncontrolled and unmonitored way. It is unclear whether ‘double bagging’ would improve the situation, as the exhibit is still required to be taken out of the packaging and then placed back in after examination and this seems likely to be the event of greatest contamination risk.

2.3.6 Conclusion

Classically in forensic science we assume that a single brown paper bag is a sufficient barrier to contamination, that we can consider the inside being free of extraneous DNA and the outside being contaminated with multiple sources of DNA from its environment. To make this assumption it is important to test various aspects of potential transfer, such as whether it is possible for DNA to be transferred from the outer surface of the bag to the inner surface (and on to the exhibit within), or from the exhibit (inner surface of the bag) to the outer surface of the bag. If the latter has occurred due to primary contact between the exhibit and the outer surface of the bag, the risk of bi-directional DNA transfer may mean that the exhibit has now been contaminated, and further examinations could result in compromised DNA profiles.

The levels of DNA detected on the exterior of evidence bags sampled within this study, reinforce the risk of exhibit contamination that is posed by contact with exhibit packaging. Much of the accumulating DNA was not able to be attributed to individuals on the laboratory elimination database or the exhibit inside of the bag. In all samples taken there was only one potential case of DNA transferring from the outer exhibit bag to the exhibit, however this may be due to the contaminating DNA being deposited in two separate primary transfer events rather than a primary transfer to the bag and then secondary to the exhibit. This indicates that (at least in the exhibits and packaging examined in this study) the risk of contaminating exhibits via the outer surface of the exhibit packing is minimal when appropriate forensic procedures are in place. If we considered this single observation out of 60 as a sample-to-sample contamination event, then this would equate to a rate of approximately 1.7%. In 13 instances, it was shown that DNA from an exhibit can be transferred to the outside of its exhibit bag, during the examination process. Again, we cannot determine whether this has occurred due to direct contact with the exhibit and the outer packaging (which would have then potentially contaminated the exhibit) or due to a secondary transfer through the examiner’s gloves (which would avoid exhibit contamination).

The study highlights the importance of assessing exhibit handling procedures, to ensure that current practices are suitable with the introduction of highly sensitive DNA profiling systems.

2.3.7 Acknowledgements

The authors would like to acknowledge the volunteers who participated in this study. We also thank FSSA staff who were involved in the collection and laboratory processing of samples.

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2.3.9 Supplementary Data

Figure 2.7. Example of an exhibit bag tapelifted in this study, with the primary sampling area shaded in red. For the alternative sampling method, the shaded top half was tapelifted before exhibit examination and the unshaded bottom half was tapelifted after exhibit examination.

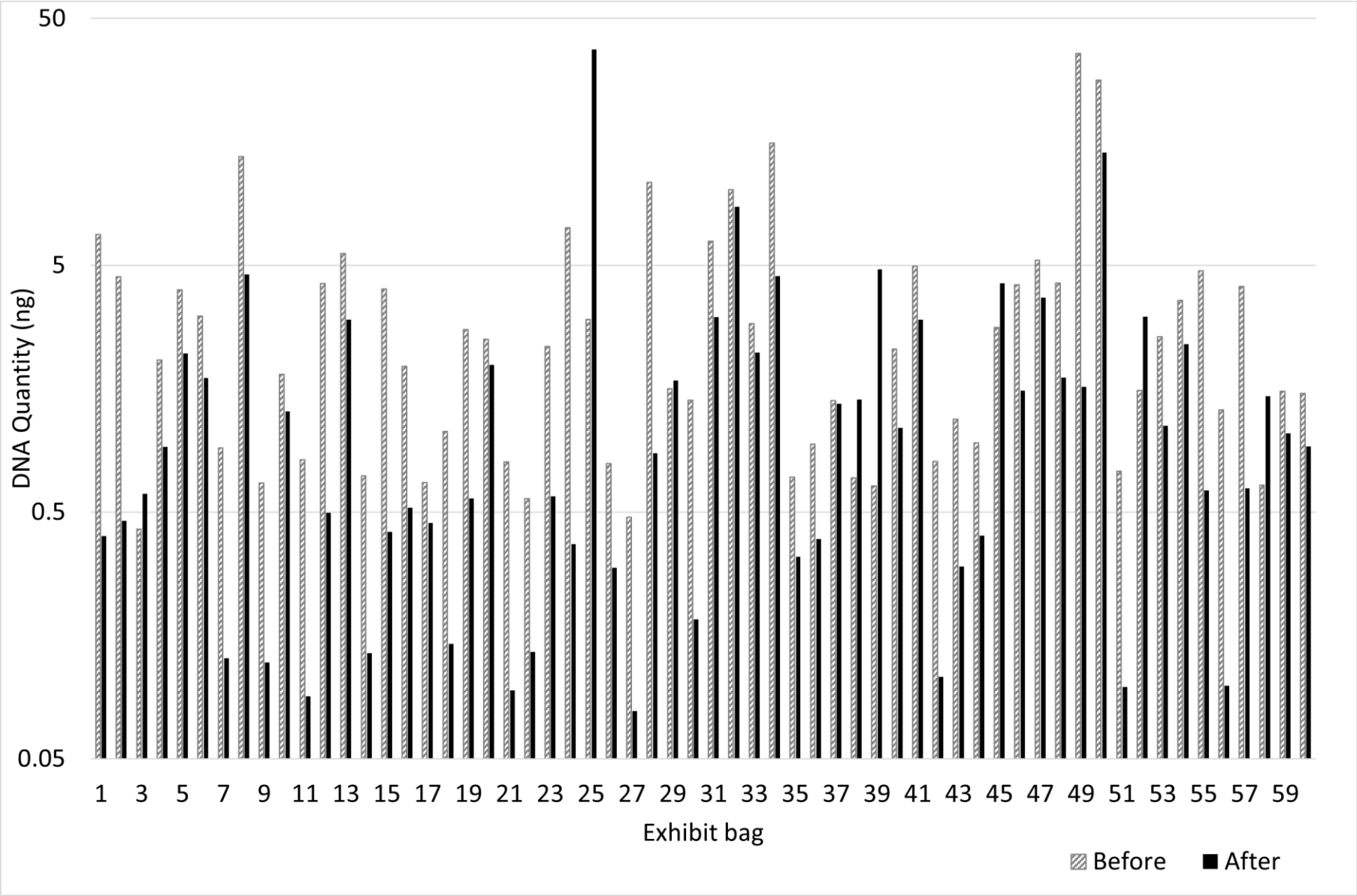

Figure 2.8. DNA Quantity (ng) recovered from individual exhibit bags sampled before and after exhibit examination.

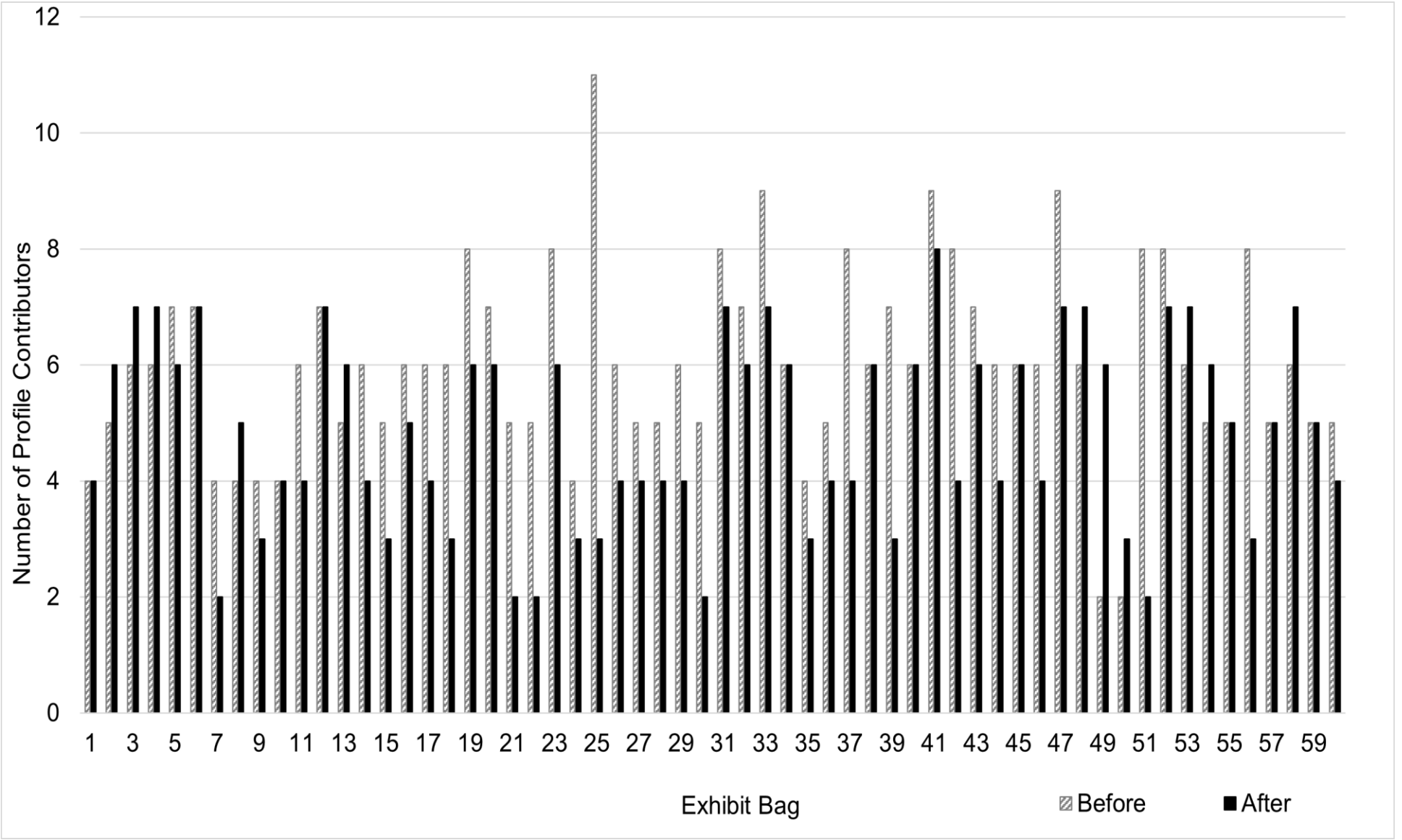

Figure 2.9. Minimum number of contributors observed in profiles generated from bags sampled before and after exhibit examination.

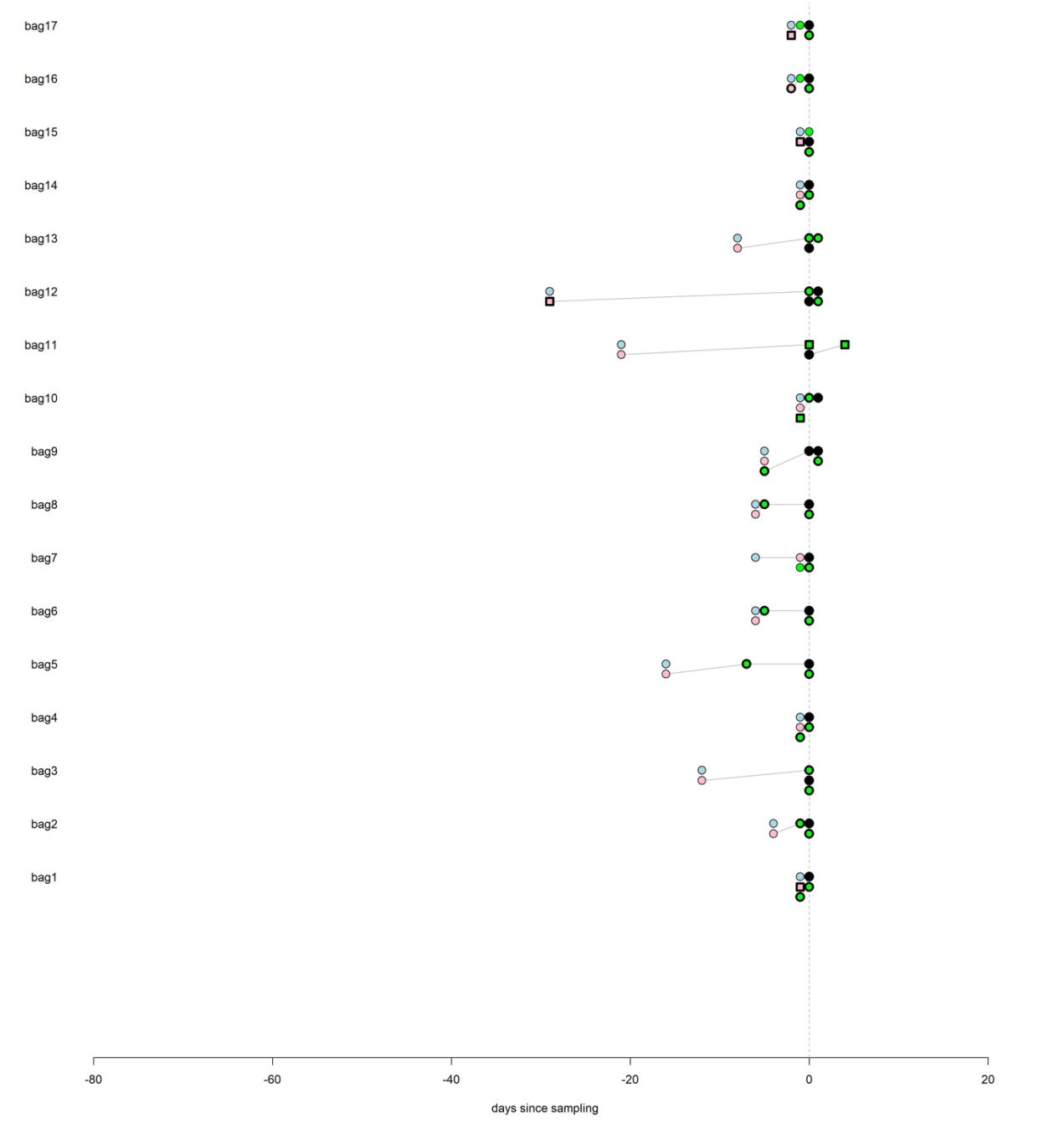

Figure 2.10. Chain of custody for bags 1-17 of the 60 exhibit bags sampled within this study. The bag number is displayed on the vertical axis and the horizontal axis represents the number of days relative to the first sample being taken from the bag. Everyone who handled the bag has been represented with a symbol. Circles indicate individuals who were identified as contacting the bag in the chain of custody but were not detected on the bag. Squares indicate individuals who were identified as contacting the bag in the chain of custody and detected on the bag either before or after. Symbols with bold borders represent individuals who provided consent to have their DNA identified. The colour of the symbols represents individuals from different departments (blue= police, red= admin, green= biology). Black circles indicate when the bag was sampled. For bags where multiple movements occurred on the same day, symbols are vertically stacked with the first movement at the top of the stack.

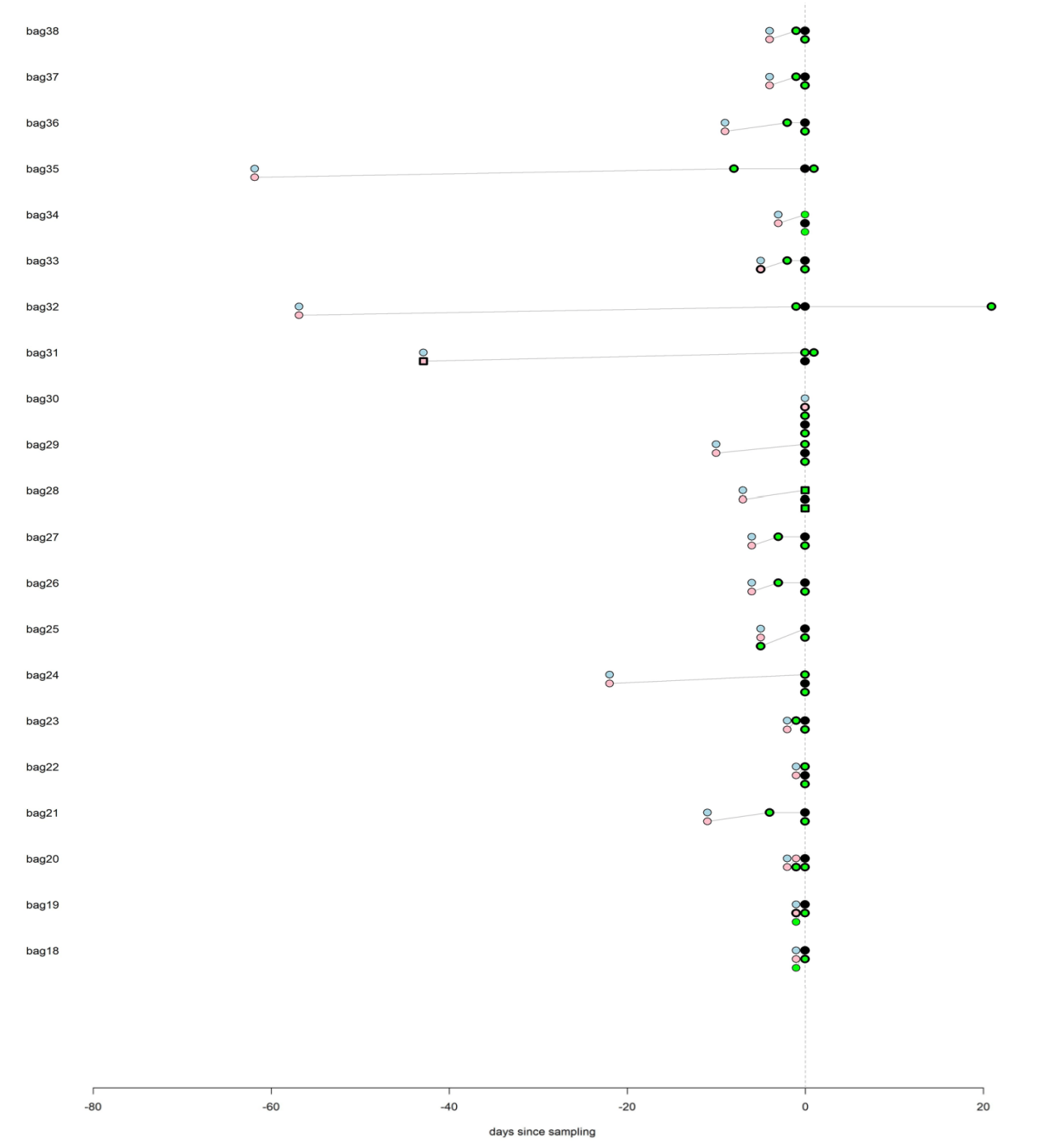

Figure 2.11 Chain of custody for bags 18-38 of the 60 exhibit bags sampled within this study. The bag number is displayed on the vertical axis and the horizontal axis represents the number of days relative to the first sample being taken from the bag. Everyone who handled the bag has been represented with a symbol. Circles indicate individuals who were identified as contacting the bag in the chain of custody but were not detected on the bag. Squares indicate individuals who were identified as contacting the bag in the chain of custody and detected on the bag either before or after. Symbols with bold borders represent individuals who provided consent to have their DNA identified. The colour of the symbols represents individuals from different departments (blue= police, red= admin, green= biology). Black circles indicate when the bag was sampled. For bags where multiple movements occurred on the same day, symbols are vertically stacked with the first movement at the top of the stack.

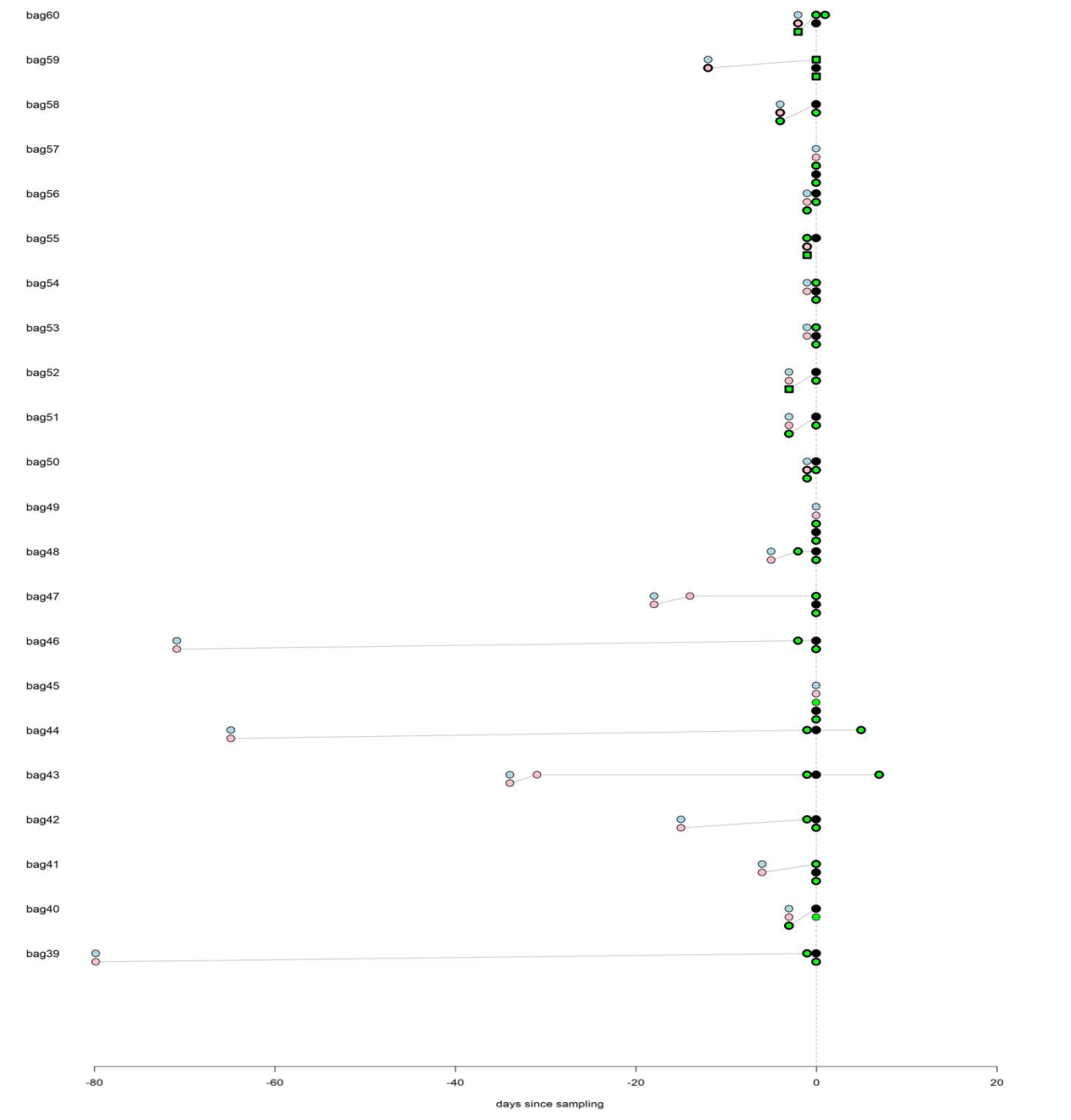

Figure 2.12 Chain of custody for bags 39-60 of the 60 exhibit bags sampled within this study. The bag number is displayed on the vertical axis and the horizontal axis represents the number of days relative to the first sample being taken from the bag. Everyone who handled the bag has been represented with a symbol. Circles indicate individuals who were identified as contacting the bag in the chain of custody but were not detected on the bag. Squares indicate individuals who were identified as contacting the bag in the chain of custody and detected on the bag either before or after. Symbols with bold borders represent individuals who provided consent to have their DNA identified. The colour of the symbols represents individuals from different departments (blue= police, red= admin, green= biology). Black circles indicate when the bag was sampled. For bags where multiple movements occurred on the same day, symbols are vertically stacked with the first movement at the top of the stack.

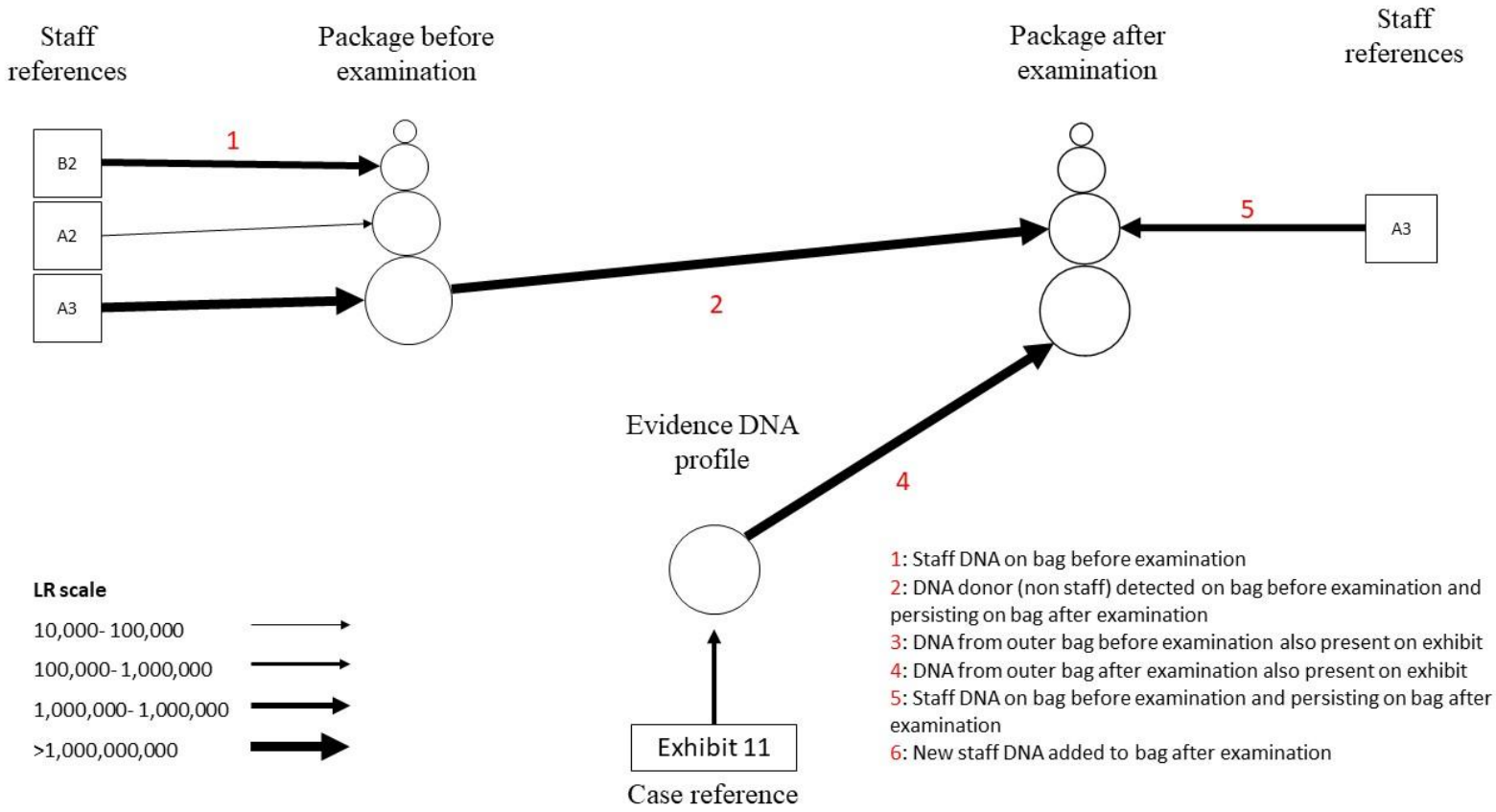

Figure 2.13. DNA transfer diagram for the package of exhibit 11. Staff matches to samples taken before or after exhibit examination have been displayed using allocated staff references, which correspond with those used in Table 2.2. and Figure 2.2. Circles represent a mixture where the number of circles represent the number of contributors to the DNA profiles generated from the bag samples and the exhibit inside of the bag. The size of each circle represents the contributor proportion. Arrows have been used to indicate DNA transfers with the direction signalled by the arrowhead. Numbers displayed in red text near arrows indicate the type of event observed, as described by the text in the bottom right corner of the figure (numbers listed here but are not present within the diagram were events which were not observed). Arrows of differing widths have been used to indicate the strength of each LR produced according to the scale displayed in the bottom left side of the figure.

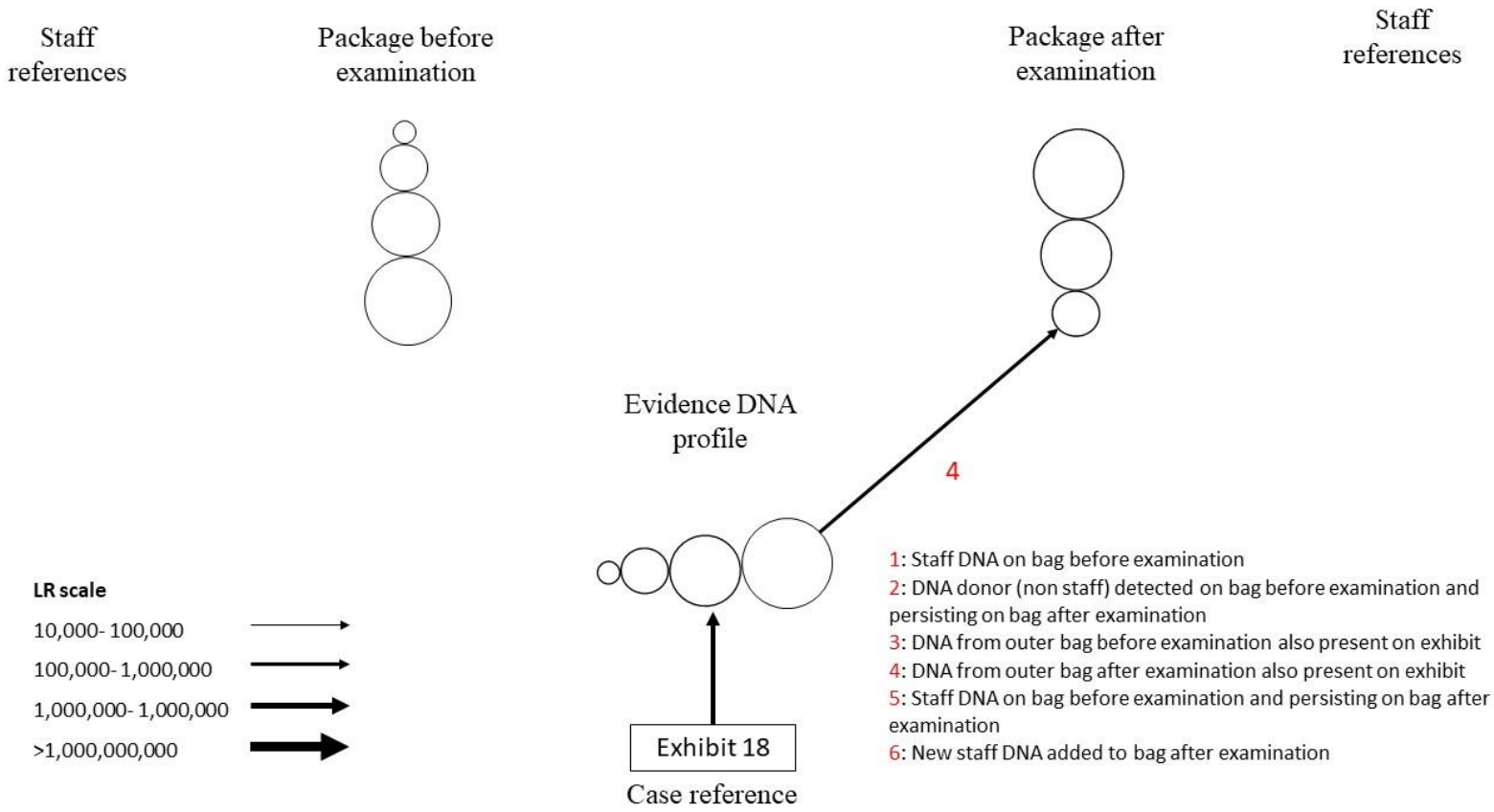

Figure 2.14. DNA transfer diagram for the package of exhibit 18. Staff matches to samples taken before or after exhibit examination have been displayed using allocated staff references, which correspond with those used in Table 2.2. and Figure 2.2. Circles represent a mixture where the number of circles represent the number of contributors to the DNA profiles generated from the bag samples and the exhibit inside of the bag. The size of each circle represents the contributor proportion. Arrows have been used to indicate DNA transfers with the direction signalled by the arrowhead. Numbers displayed in red text near arrows indicate the type of event observed, as described by the text in the bottom right corner of the figure (numbers listed here but are not present within the diagram were events which were not observed). Arrows of differing widths have been used to indicate the strength of each LR produced according to the scale displayed in the bottom left side of the figure.

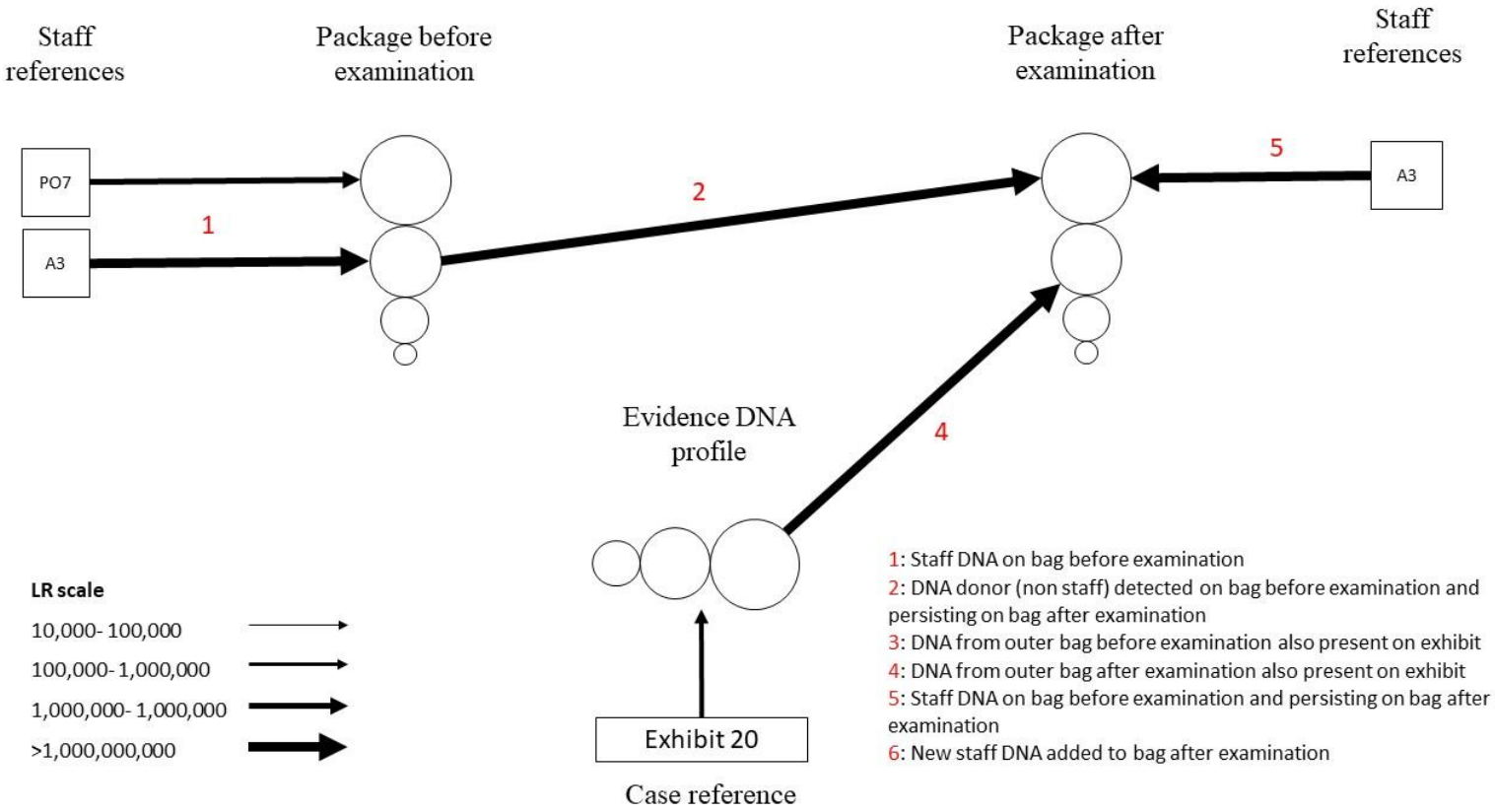

Figure 2.15. DNA transfer diagram for the package of exhibit 20. Staff matches to samples taken before or after exhibit examination have been displayed using allocated staff references, which correspond with those used in Table 2.2. and Figure 2.2. Circles represent a mixture where the number of circles represent the number of contributors to the DNA profiles generated from the bag samples and the exhibit inside of the bag. The size of each circle represents the contributor proportion. Arrows have been used to indicate DNA transfers with the direction signalled by the arrowhead. Numbers displayed in red text near arrows indicate the type of event observed, as described by the text in the bottom right corner of the figure (numbers listed here but are not present within the diagram were events which were not observed). Arrows of differing widths have been used to indicate the strength of each LR produced according to the scale displayed in the bottom left side of the figure.

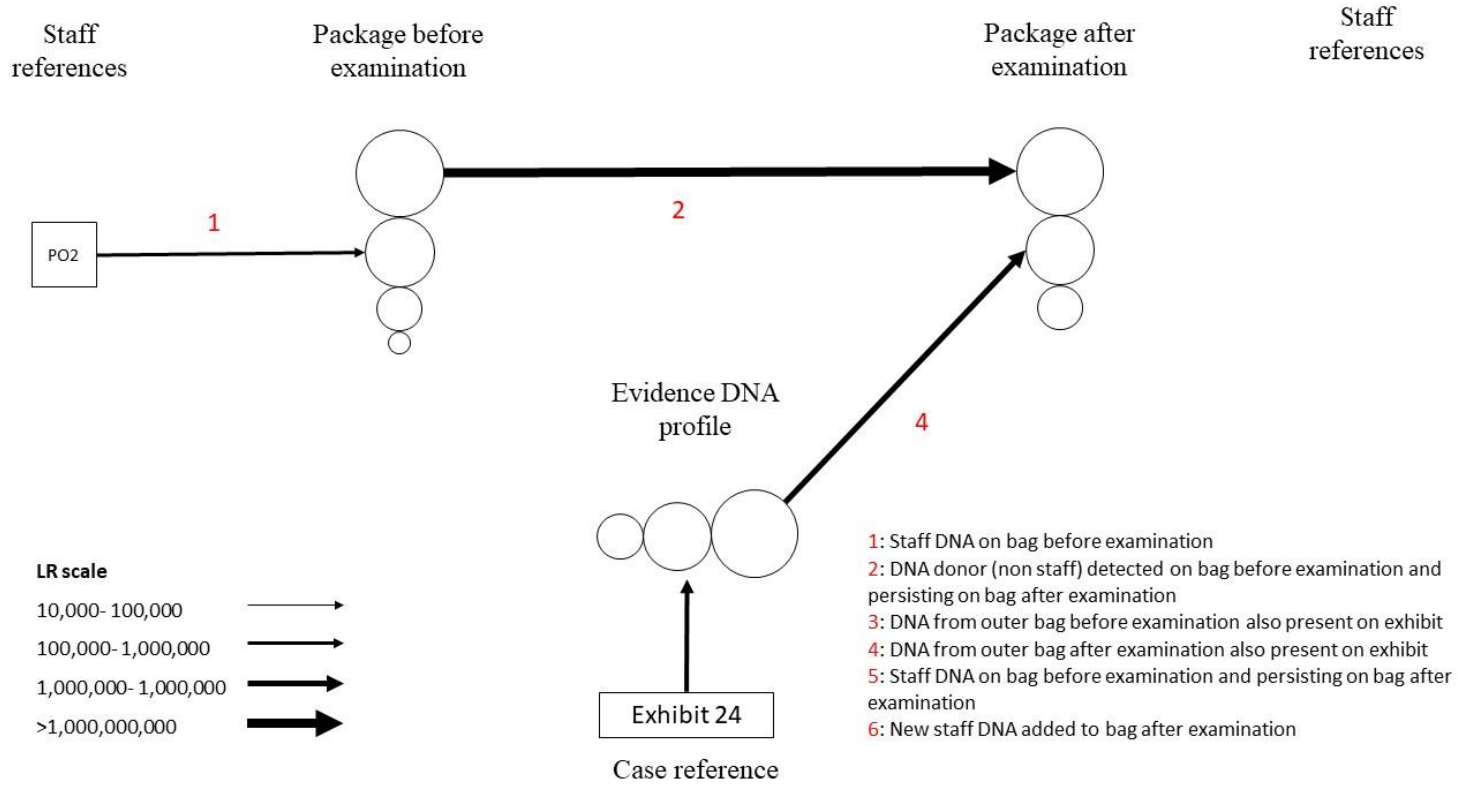

Figure 2.16. DNA transfer diagram for the package of exhibit 24. Staff matches to samples taken before or after exhibit examination have been displayed using allocated staff references, which correspond with those used in Table 2.2. and Figure 2.2. Circles represent a mixture where the number of circles represent the number of contributors to the DNA profiles generated from the bag samples and the exhibit inside of the bag. The size of each circle represents the contributor proportion. Arrows have been used to indicate DNA transfers with the direction signalled by the arrowhead. Numbers displayed in red text near arrows indicate the type of event observed, as described by the text in the bottom right corner of the figure (numbers listed here but are not present within the diagram were events which were not observed). Arrows of differing widths have been used to indicate the strength of each LR produced according to the scale displayed in the bottom left side of the figure.

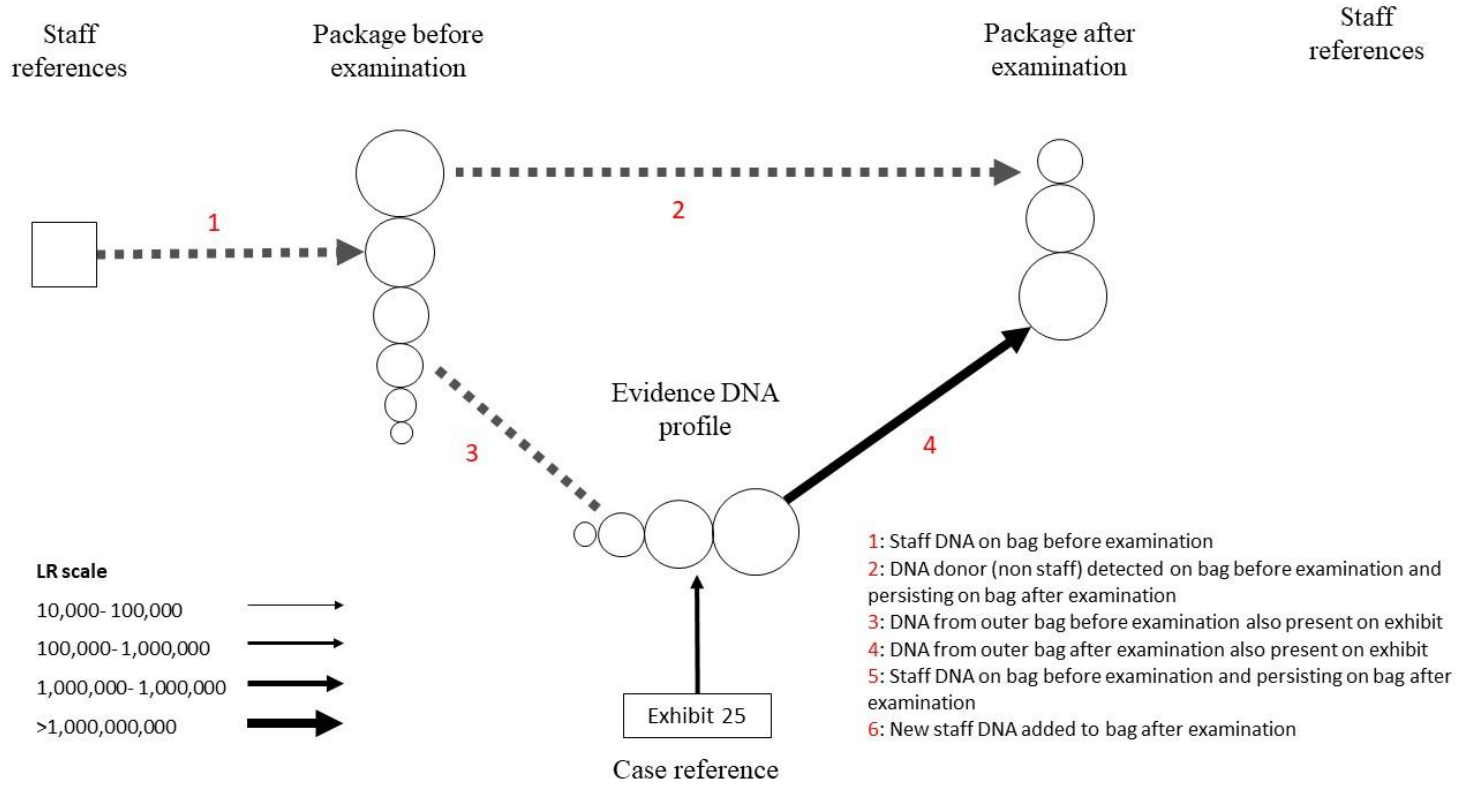

Figure 2.17. DNA transfer diagram for the package of exhibit 25. Staff matches to samples taken before or after exhibit examination have been displayed using allocated staff references, which correspond with those used in Table 2.2. and Figure 2.2. Circles represent a mixture where the number of circles represent the number of contributors to the DNA profiles generated from the bag samples and the exhibit inside of the bag. The size of each circle represents the contributor proportion. Arrows have been used to indicate DNA transfers with the direction signalled by the arrowhead. Numbers displayed in red text near arrows indicate the type of event observed, as described by the text in the bottom right corner of the figure (numbers listed here but are not present within the diagram were events which were not observed). Arrows of differing widths have been used to indicate the strength of each LR produced according to the scale displayed in the bottom left side of the figure. Dashed arrows indicate that a type of event is unknown, as one of the samples taken from the bag produced a profile too complex for analysis.

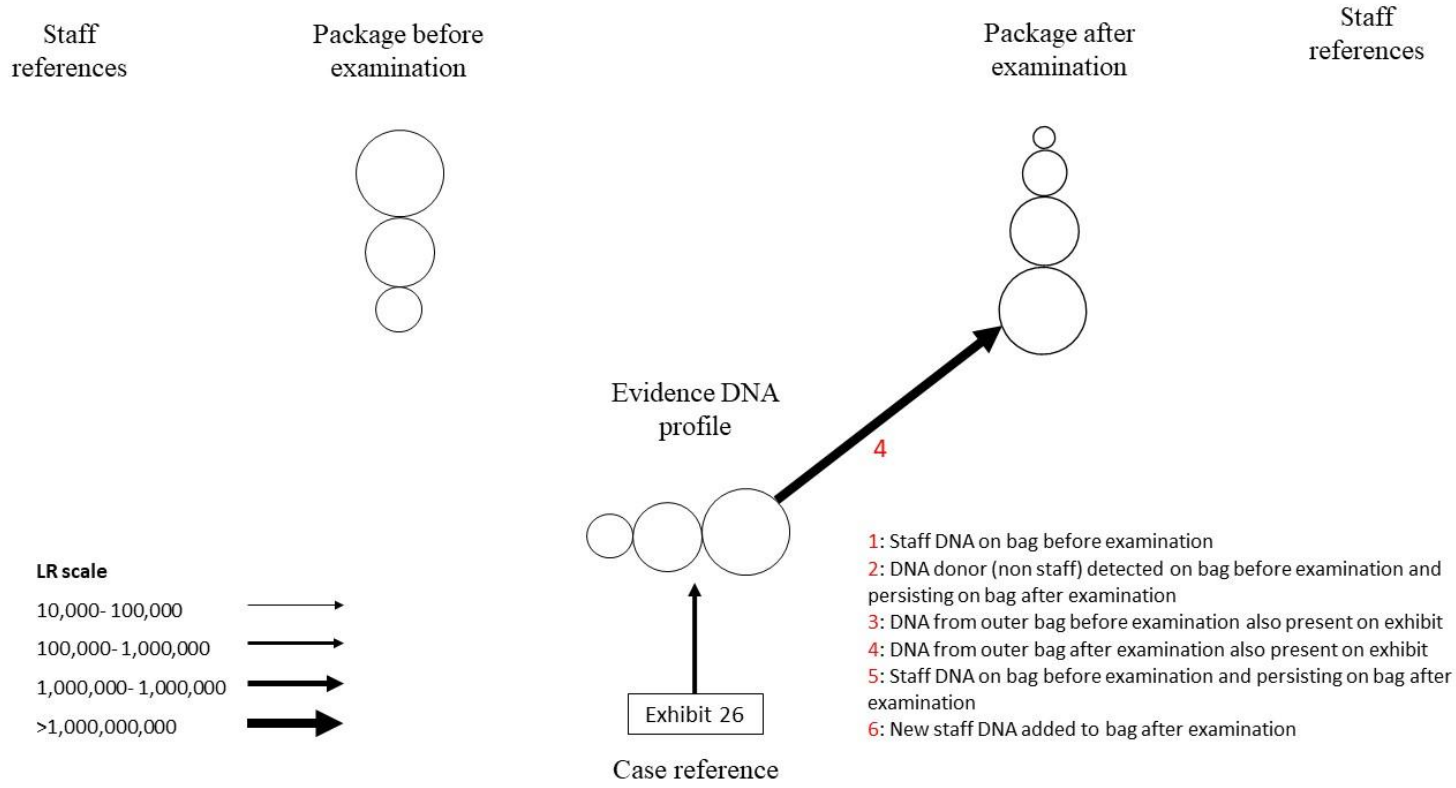

Figure 2.18. DNA transfer diagram for the package of exhibit 26. Staff matches to samples taken before or after exhibit examination have been displayed using allocated staff references, which correspond with those used in Table 2.2. and Figure 2.2. Circles represent a mixture where the number of circles represent the number of contributors to the DNA profiles generated from the bag samples and the exhibit inside of the bag. The size of each circle represents the contributor proportion. Arrows have been used to indicate DNA transfers with the direction signalled by the arrowhead. Numbers displayed in red text near arrows indicate the type of event observed, as described by the text in the bottom right corner of the figure (numbers listed here but are not present within the diagram were events which were not observed). Arrows of differing widths have been used to indicate the strength of each LR produced according to the scale displayed in the bottom left side of the figure.

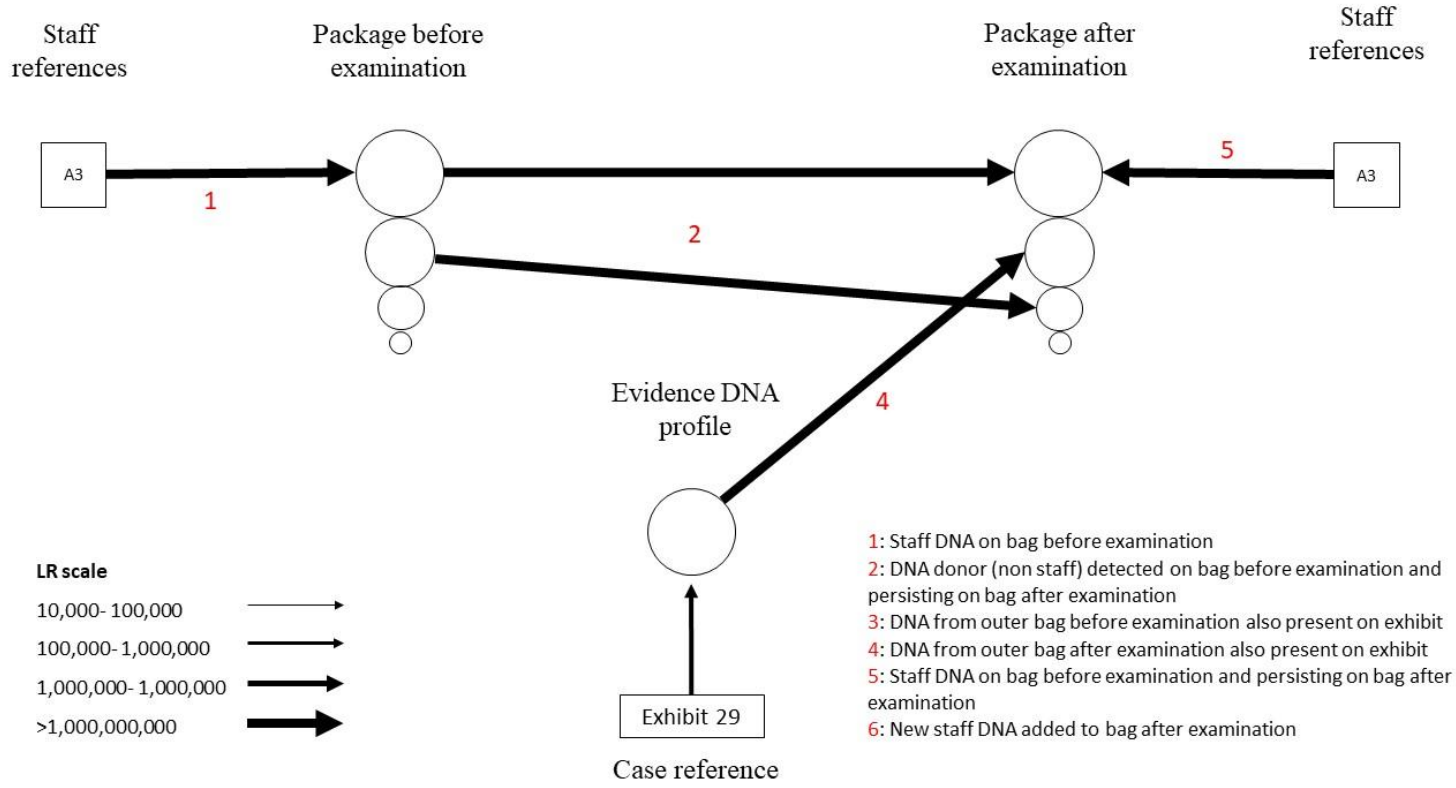

Figure 2.19. DNA transfer diagram for the package of exhibit 29. Staff matches to samples taken before or after exhibit examination have been displayed using allocated staff references, which correspond with those used in Table 2.2. and Figure 2.2. Circles represent a mixture where the number of circles represent the number of contributors to the DNA profiles generated from the bag samples and the exhibit inside of the bag. The size of each circle represents the contributor proportion. Arrows have been used to indicate DNA transfers with the direction signalled by the arrowhead. Numbers displayed in red text near arrows indicate the type of event observed, as described by the text in the bottom right corner of the figure (numbers listed here but are not present within the diagram were events which were not observed). Arrows of differing widths have been used to indicate the strength of each LR produced according to the scale displayed in the bottom left side of the figure.

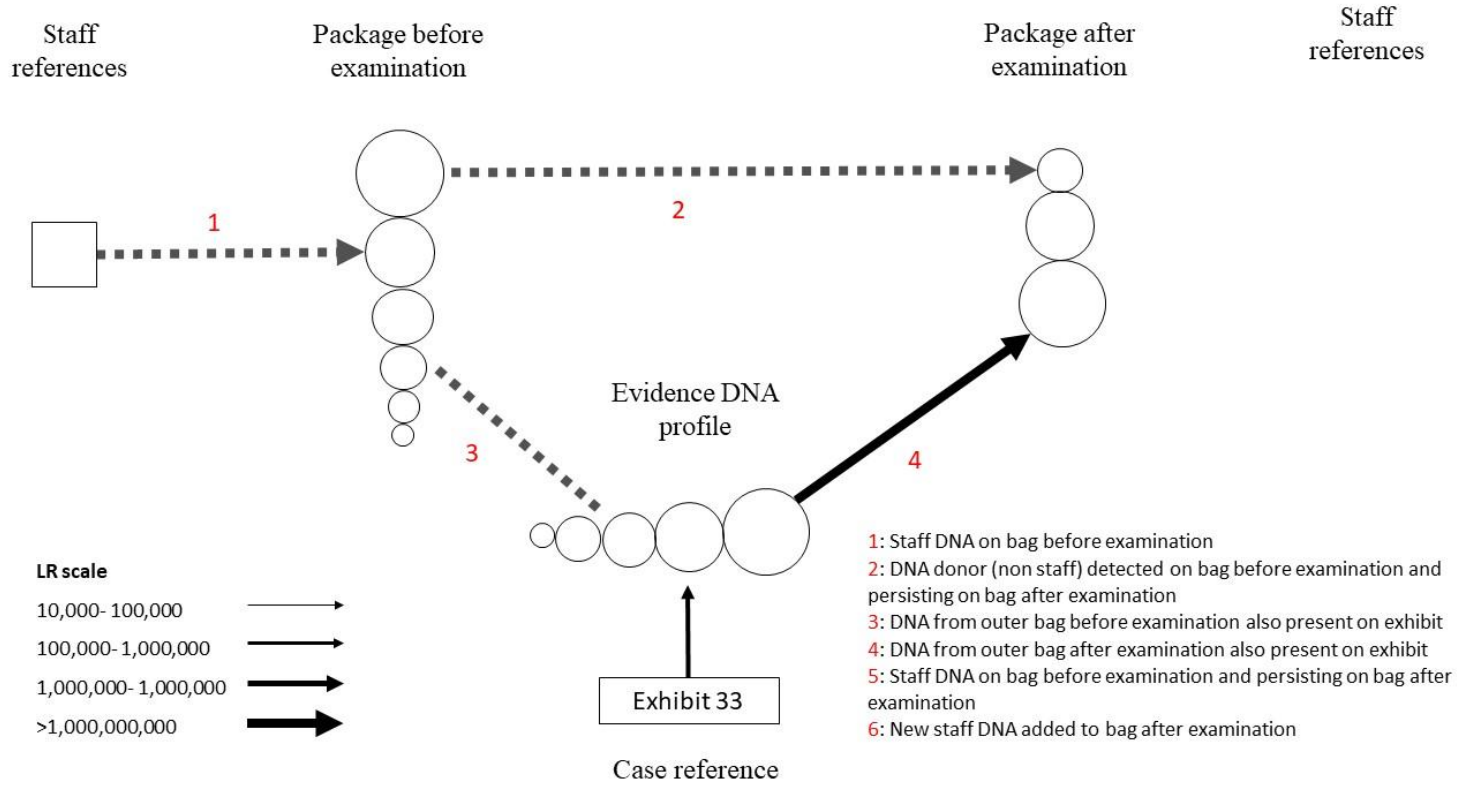

Figure 2.20. DNA transfer diagram for the package of exhibit 33. Staff matches to samples taken before or after exhibit examination have been displayed using allocated staff references, which correspond with those used in Table 2.2. and Figure 2.2. Circles represent a mixture where the number of circles represent the number of contributors to the DNA profiles generated from the bag samples and the exhibit inside of the bag. The size of each circle represents the contributor proportion. Arrows have been used to indicate DNA transfers with the direction signalled by the arrowhead. Numbers displayed in red text near arrows indicate the type of event observed, as described by the text in the bottom right corner of the figure (numbers listed here but are not present within the diagram were events which were not observed). Arrows of differing widths have been used to indicate the strength of each LR produced according to the scale displayed in the bottom left side of the figure. Dashed arrows indicate that a type of event is unknown, as one of the samples taken from the bag produced a profile too complex for analysis.

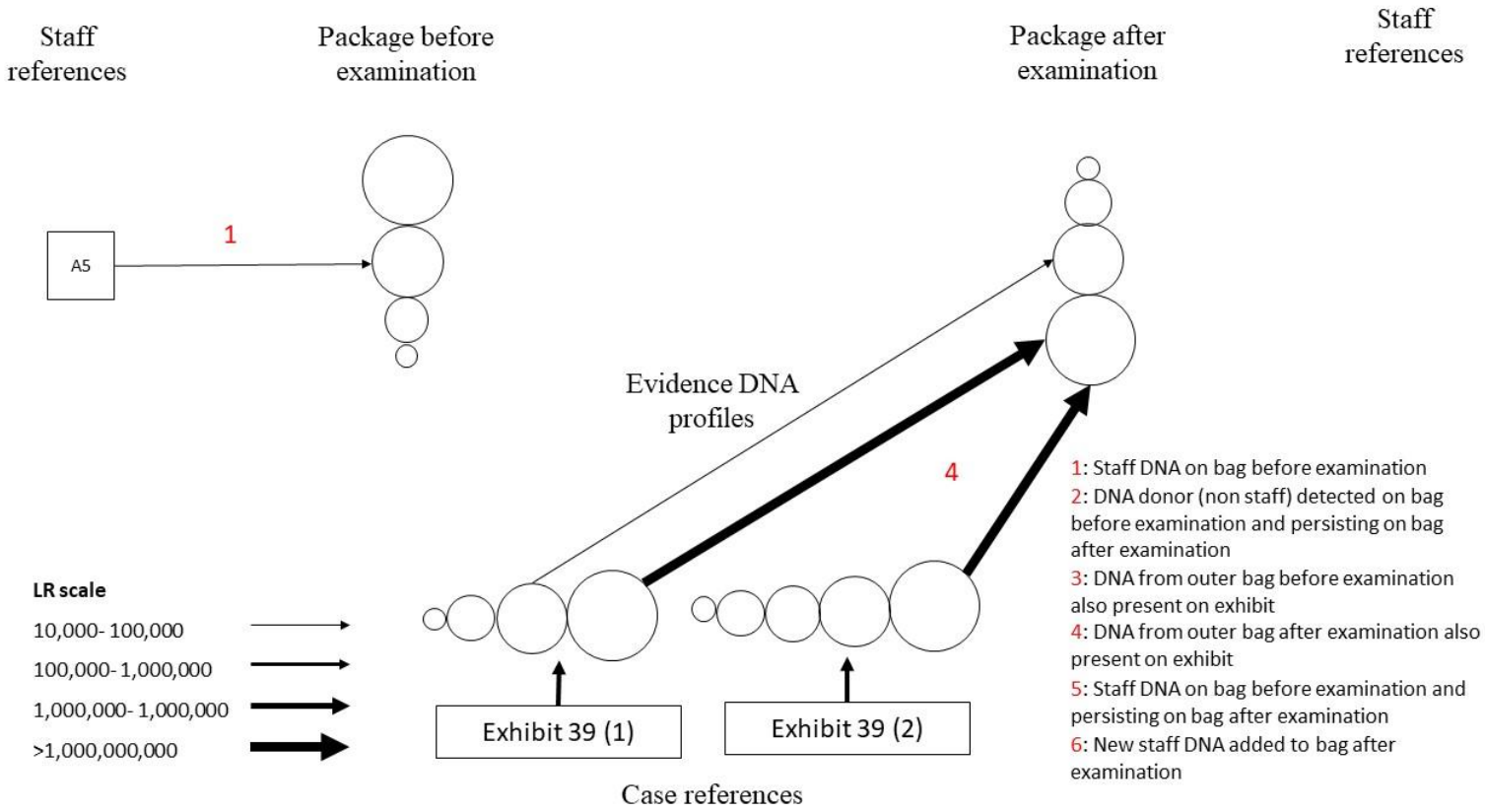

Figure 2.21. DNA transfer diagram for the package of exhibit 39. Staff matches to samples taken before or after exhibit examination have been displayed using allocated staff references, which correspond with those used in Table 2.2. and Figure 2.2. Circles represent a mixture where the number of circles represent the number of contributors to the DNA profiles generated from the bag samples and the exhibit inside of the bag. The size of each circle represents the contributor proportion. Arrows have been used to indicate DNA transfers with the direction signalled by the arrowhead. Numbers displayed in red text near arrows indicate the type of event observed, as described by the text in the bottom right corner of the figure (numbers listed here but are not present within the diagram were events which were not observed). Arrows of differing widths have been used to indicate the strength of each LR produced according to the scale displayed in the bottom left side of the figure.

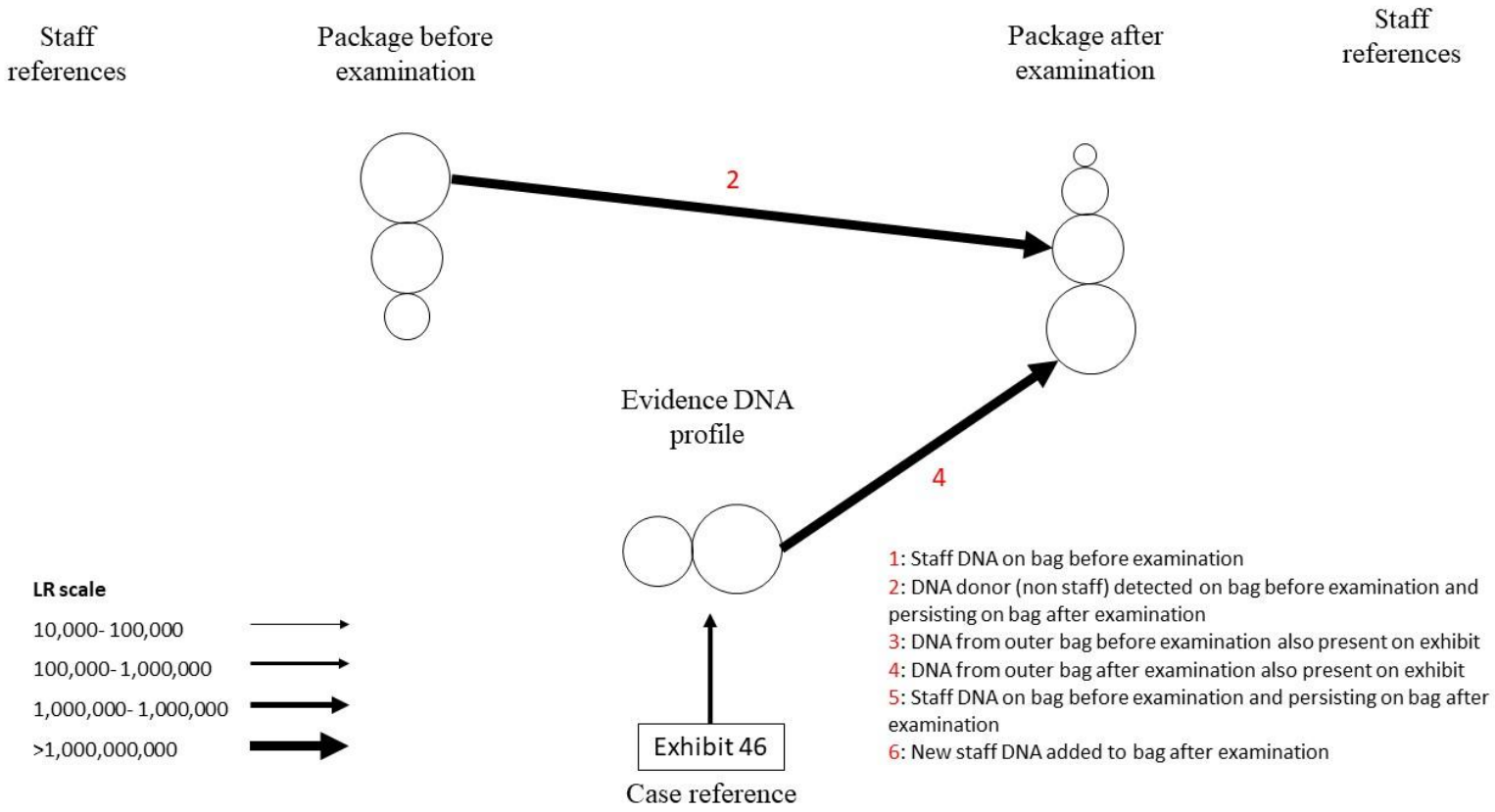

Figure 2.22. DNA transfer diagram for the package of exhibit 46. Staff matches to samples taken before or after exhibit examination have been displayed using allocated staff references, which correspond with those used in Table 2.2. and Figure 2.2. Circles represent a mixture where the number of circles represent the number of contributors to the DNA profiles generated from the bag samples and the exhibit inside of the bag. The size of each circle represents the contributor proportion. Arrows have been used to indicate DNA transfers with the direction signalled by the arrowhead. Numbers displayed in red text near arrows indicate the type of event observed, as described by the text in the bottom right corner of the figure (numbers listed here but are not present within the diagram were events which were not observed). Arrows of differing widths have been used to indicate the strength of each LR produced according to the scale displayed in the bottom left side of the figure.

Figure 2.23. DNA transfer diagram for the package of exhibit 51. Staff matches to samples taken before or after exhibit examination have been displayed using allocated staff references, which correspond with those used in Table 2.2. and Figure 2.2. Circles represent a mixture where the number of circles represent the number of contributors to the DNA profiles generated from the bag samples and the exhibit inside of the bag. The size of each circle represents the contributor proportion. Arrows have been used to indicate DNA transfers with the direction signalled by the arrowhead. Numbers displayed in red text near arrows indicate the type of event observed, as described by the text in the bottom right corner of the figure (numbers listed here but are not present within the diagram were events which were not observed). Arrows of differing widths have been used to indicate the strength of each LR produced according to the scale displayed in the bottom left side of the figure.

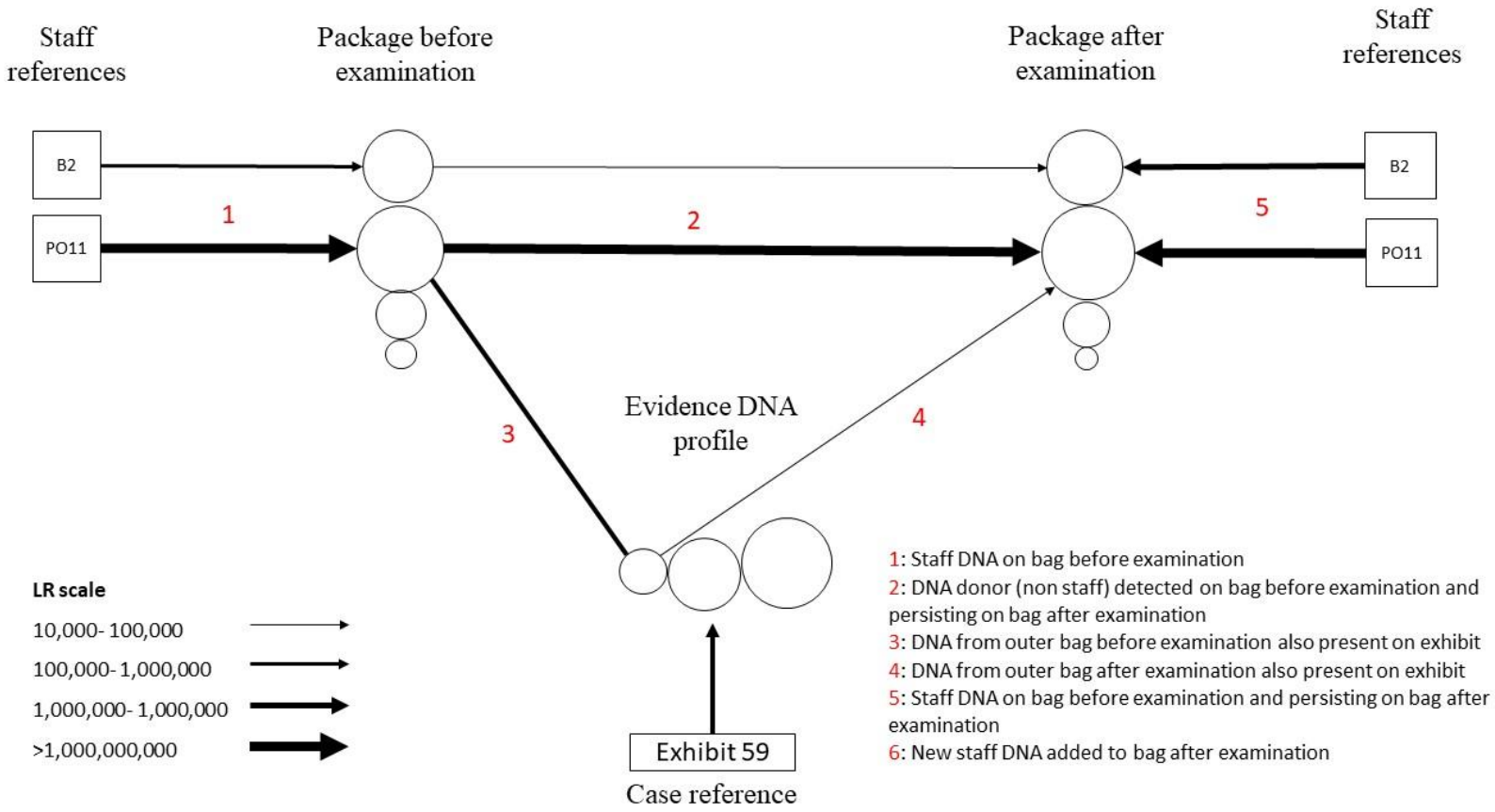

Figure 2.24. DNA transfer diagram for the package of exhibit 59. Staff matches to samples taken before or after exhibit examination have been displayed using allocated staff references, which correspond with those used in Table 2.2. and Figure 2.2. Circles represent a mixture where the number of circles represent the number of contributors to the DNA profiles generated from the bag samples and the exhibit inside of the bag. The size of each circle represents the contributor proportion. Arrows have been used to indicate DNA transfers with the direction signalled by the arrowhead. Numbers displayed in red text near arrows indicate the type of event observed, as described by the text in the bottom right corner of the figure (numbers listed here but are not present within the diagram were events which were not observed). Arrows of differing widths have been used to indicate the strength of each LR produced according to the scale displayed in the bottom left side of the figure.

CHAPTER 3 : DNA WITHIN FORENSIC WORKSPACES

3.1 Preface

While there are a number of studies which detail the levels of environmental DNA present within office workspaces and laboratories, the accumulation of DNA within forensic exhibit storage areas has not previously been studied. Due to the lack of research of this nature, combined with the limited research involving exhibit packaging, the risk that DNA accumulating within exhibit storage locations poses to the integrity of DNA evidence is unknown. The research within this chapter provides insight into the levels of DNA accumulating on workspaces within environments where exhibits are handled and stored.

Shelves within the exhibit storeroom of an operational forensic laboratory were sampled over a 14 week time period, to determine the level of DNA accumulation and transfer which occurs within this environment. Additionally, the source of accumulating DNA was explored, through the comparison of DNA profiles from samples, forensic staff and those generated from the exhibits which were stored upon the shelves sampled across the duration of the study. Results are reported within a manuscript which was accepted for publication in Forensic Science International: Genetics, 62:102799. https://doi.org/10.1016/j.fsigen.2022.102799

3.2 Statement of authorship

Author	Contribution (%)			Signature	Date
	Research Design	Data collection and analysis	Writing and editing		
Claire Mercer	**85**	**80**	**70**	**Claire Mercer**	**7/11/2023**
Duncan Taylor	5	10	10	Duncan Taylor	7/11/2023
Julianne Henry	5	5	10	Julianne Henry	7/11/2023
Adrian Linacre	5	5	10	Adrian Linacre	24/10/2023

3.3 Manuscript: DNA accumulation and transfer within an operational forensic exhibit storeroom

Claire Mercer, Duncan Taylor, Julianne Henry, Adrian Linacre. DNA accumulation and transfer within an operational forensic exhibit storeroom. Forensic Science International Genetics (2023) 62:102799.

3.3.1 Abstract

The increased sensitivity of current DNA profiling technologies allows the detection of trace amounts of DNA. With these advancements, there is an increased probability of detecting trace levels of DNA from contamination. Studies which investigate the accumulation and transfer of DNA within forensic laboratories provide insight into the possible mechanisms which may result in the contamination of exhibits. To gain a greater understanding of the level of DNA transfer between exhibit packaging and forensic workspaces, the accumulation of DNA within an operational forensic exhibit storeroom was investigated. Samples were collected from previously cleaned forensic exhibit storeroom shelves at various time points over a 14-week period. To determine the source of accumulating DNA, profiles generated from shelf samples were compared to the laboratory staff elimination database and the profiles generated from exhibits stored on each of the shelves sampled over the course of the study. Additionally, all samples were compared using STRmix™ mixture-to-mixture profile analysis, to identify the presence of common non-staff DNA donors and DNA from exhibits stored on the shelves sampled. As sampling time intervals increased, there was a significant increase in DNA quantity (ng) and number of profile contributors. The shelf height was also observed to influence the number of profile contributors, with higher numbers of contributors being found on lower shelves. DNA profiles generated from the shelf samples were matched to DNA from forensic staff members who enter the storeroom and police employees, who do not enter the storeroom. There were three instances where a common DNA profile contributor was identified between a shelf sample and the profile generated from an exhibit.

This study provides insight into whether current exhibit storage procedures are still adequate given the highly sensitive DNA profiling systems currently used.

3.3.2 Introduction

With the introduction of highly sensitive DNA technologies that can detect very small amounts of genetic material, there is an increase in the number of possible transfer mechanisms that may explain how DNA came to be on a surface. As the focus of DNA evidence in court moves from questioning the source of the DNA, to addressing the possible mechanism of deposition [1], research which investigates DNA transfer, persistence, prevalence and recovery (TPPR) has become increasingly important [2].

Many studies demonstrate the propensity for DNA to be directly transferred to a surface [3-6] and then further transferred indirectly, through subsequent contacts with other substrates [7-12]. In previous research, primary, secondary, tertiary and quaternary transfer has been shown to occur within a variety of scenarios involving numerous substrates [3, 4, 7, 9, 10, 12-14]. It is also known that DNA can persist on surfaces for extended durations, as demonstrated by the detection of DNA which has persisted on an item for several years in numerous cold cases [15-17]. The persistence of DNA is highly dependent on environmental conditions [18-20], with substrates kept indoors within dark, temperature controlled environments providing the most favourable conditions [18, 19, 21, 22].

The ability to detect 'trace' amounts of DNA comes with the consequence of an increased chance of detecting material which has been transferred to an exhibit during the forensic process and is not related to an alleged event. Contamination can have severe impacts on the outcome of criminal investigations [23, 24]. Accredited forensic laboratories implement strict procedures to minimise and detect contamination [25-27]. Personal protective equipment (PPE) is used within laboratories where samples and exhibits are processed to minimise the risk of person-to-sample contamination. Frequent and thorough cleaning of surfaces and equipment within these areas also reduces the risk of contamination [25, 27]. To ensure that cleaning procedures are effective, some laboratories additionally monitor background DNA levels within sensitive areas [28-30]. To enable the detection of staff contamination, it is routine for forensic laboratories to compare samples processed to an elimination database containing DNA profiles of forensic staff and crime scene personnel [26]. Despite the existence of strict contamination minimisation procedures, forensic laboratories still encounter contamination [12, 23, 31, 32].

There are many stages throughout the forensic process where the opportunity for exhibit contamination is known to exist [26, 27, 31, 33-38]. Previous studies indicate that there is a contamination risk posed by DNA which accumulates on workspaces and equipment within areas where exhibits are handled [12, 28, 31, 35, 39, 40]. There is an abundance of research which investigates DNA accumulation and transfer within sensitive areas where exhibits are examined and samples are processed [35, 39, 41-43]. There are also several studies which investigate the accumulation and transfer of DNA within non-sensitive areas, [29, 44, 45]. Research which explores DNA accumulation in such environments provides knowledge of background DNA levels within workspaces and the ability for individuals to deposit DNA within areas they frequent [28-30, 44-47]. In a study which investigated DNA transfer across the Biology department of Forensic Science SA (FSSA), it was determined that DNA was most likely to be found in an individual's immediate areas, but could also be detected in areas where they had spent limited time and had made contact with objects [29]. The amount of DNA deposited by different individuals was found to vary [29]. Similar findings were yielded in another study, which investigated the DNA transfer between temporary and original users of an office space [46]. Even after temporary use by another

individual, DNA from most frequent workspace users were found to be major profile contributors [46]. In instances where DNA from a temporary user was detected, it was usually on items the individual had contacted [46]. In another study, it was observed that DNA from an individual could accumulate on nearby surfaces in their immediate surroundings after a single day, without direct contact with the substrate [45]. An individual speaking, coughing or sneezing near the substrate may explain such DNA accumulation [32, 48, 49]. Aerial DNA transfer may also explain the accumulation of an individual's DNA on a surface, which has not been directly contacted. Previous studies indicate that there is the potential for saliva droplets from speaking and coughing to be transmitted through the air [50, 51] and remain airborne for several days [52]. Within another study, it was determined that human DNA could be detected in samples taken using an air collection device, through the identification of 12S and 16S DNA fragments [53]. While this research provides proof of the concept of detecting human DNA in air samples [53], there is currently limited research which involves collecting human DNA from the air for use in forensic studies [54].

While there are numerous studies which investigate DNA transfer within examination laboratories [28, 30] and other workspaces [29, 45, 46], there is limited research which explores DNA transfer within exhibit storage locations. It has previously been demonstrated that DNA can accumulate on the exterior of evidence bags during storage within an exhibit storeroom [55]. The source of the DNA, which was observed to accumulate on the exterior of exhibit bags, was not determined, however possible explanations include DNA transferred through contact with other exhibit packages and shelves or forensic staff who work within the area [55]. It was also demonstrated that during the initial exhibit packaging and examination, DNA from an exhibit can be added to the exterior of its package [55]. In another study, which investigated DNA that accumulates on exhibit packaging, there were instances where DNA from an exhibit was detected on the exterior of its package after examination [56]. The detection of exhibit-related DNA on the exterior of evidence bags raises the question of whether such DNA can be further transferred indirectly to other exhibit bags, workspaces and exhibit storage locations. As there is the ability for DNA to be transferred from the exterior of an exhibit bag to the exhibit within [31], such indirect transfer is of concern, as it creates the potential for cross-contamination between exhibits (both within and between cases depending on how exhibits are stored). While exhibit packaging is known to be an efficient DNA transfer vector [31, 36, 55, 56], there is limited data regarding the transfer of DNA between exhibit packaging and other substrates. The risk that DNA accumulation and transfer within exhibit storage locations may pose to the integrity of DNA evidence is unknown and further research is required.

This study aimed to provide a better understanding of the level of DNA transfer that occurs between exhibit packaging and forensic workspaces through sampling shelves within the FSSA exhibit storeroom throughout a 14 week period. The level of DNA which accumulates within exhibit storage locations, and the source of accumulating DNA, was investigated. At FSSA exhibits are stored on shelves within steel compactus storage units, within a secure storeroom. The compactus

units are fitted with wheeled traction systems that allow units to be closely packed together and moved apart when access to the unit is required. Due to the variation in the size and quantity of exhibits stored on each shelf, the nature of contact between exhibits also varies. Some shelves contain many exhibits, which are packed together and constantly in direct contact with other exhibits, while other shelves contain few exhibits which are loosely packed and have little contact with other exhibits. Exhibits relating to all departments of FSSA are stored within the exhibit storeroom. Exhibits remain enclosed in sealed packaging within the storeroom until they are collected for examination by an individual from the relevant department. Exhibits containing biological material are individually packaged within a single paper evidence bag or envelope. In addition to paper bags, other packaging, including plastic bags, metal tins and cardboard tubes, may be used to package exhibits that do not contain biological material. Access to the storeroom is restricted to FSSA staff and the area is not routinely cleaned. Wearing of PPE is not mandatory to access the storeroom or handle exhibit packaging. While general exhibit storage guidelines are likely to be similar between forensic laboratories, each adheres to its own protocols. Investigating the transfer of DNA within areas where exhibits are handled and stored, provides insight into whether current storage procedures are adequate given the sensitivity of modern DNA profiling technologies.

3.3.3 Materials and methods

3.3.3.1 Sample collection

To investigate the level of DNA accumulating within forensic exhibit storage locations, samples were taken from compactus storage units within the FSSA exhibit storeroom over a 14-week period. There are a total of 20 compactus units within the FSSA exhibit storeroom which consist of six shelves. A top, middle, and bottom shelf from seven randomly chosen compactus units was selected for sampling. To simplify the description of sampling locations, the compactus units selected for sampling are indicated in the floorplan in Figure 3.1.

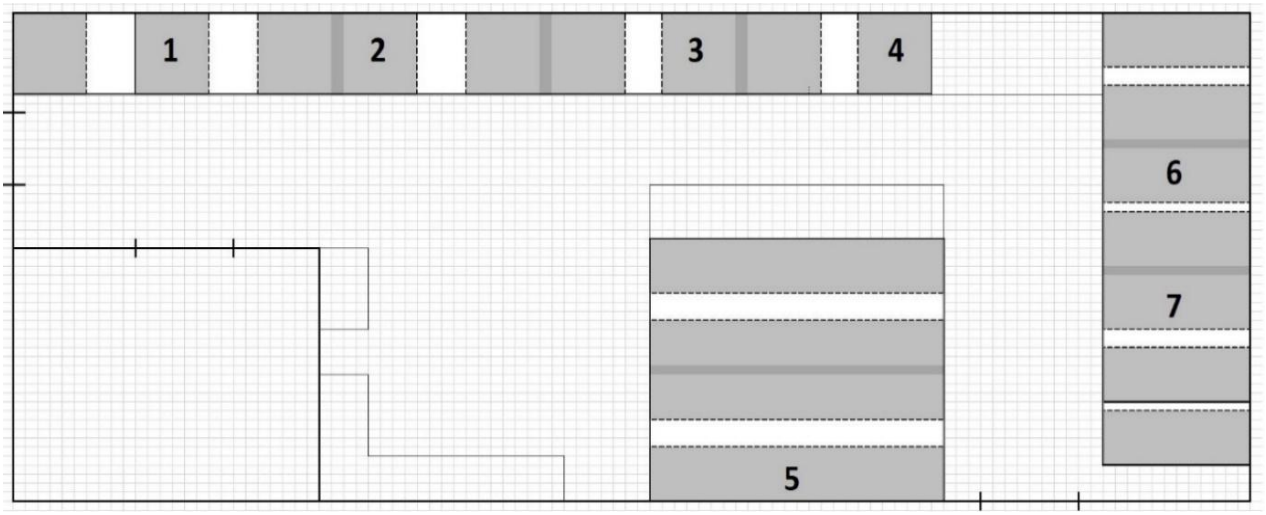

Figure 3.1. Floorplan of the FSSA exhibit storeroom. Areas shaded in grey represent movable steel compactus storage units, with the accessible side of the compactus unit indicated with a dashed line. Thick dark grey shaded lines have been used to indicate when the rear of two compactus units are joined together. The compactus units sampled have been indicated using numbers 1 - 7. Thin black lines represent the perimeter of storage fixtures and other furniture, and thick black lines indicate walls, with the position of doors marked along the line using two perpendicular lines. The approximate dimensions of the storeroom are 10 m by 5 m.

At the beginning of the study all exhibits were removed from the selected shelves and the front portion of the shelf was divided into seven 11 x 15 cm sampling sections. The whole shelf surface was thoroughly cleaned using a 0.5 % sodium hypochlorite solution, followed by distilled water. All exhibits were placed back onto the shelf, without directly contacting the marked sampling areas. One section of all shelves was swabbed immediately after exhibits were placed back onto to the shelf, to ensure that the cleaning process was effective. All samples were taken using Isohelix™ DNA/RNA Buccal Swabs (Cell Projects Ltd., Harrietsham, United Kingdom) moistened with isopropanol, which were rubbed across the whole area of the designated sampling section. A new section (adjacent to the area previously sampled at each time point) was swabbed on all shelves 1 day and 1, 2, 4, 8 and 14 weeks after cleaning. All samples were taken by an individual wearing a face mask, laboratory coat, hair net and gloves. To determine a baseline level of DNA accumulating over time, two compactus units (labelled 4 and 5 in Figure 3.1) did not contain any exhibits for the duration of the study.

3.3.3.2 DNA analysis

All stages of DNA analyses were performed using in-house validated protocols at FSSA. DNA samples were extracted using the DNA IQ system (Promega, Madison, WI, USA) on a Microlab® AutoLys STAR workstation (Hamilton Company, Reno, NV, USA), with an elution volume of 60 µL. DNA yields were quantified using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific, Waltham, MA, USA) on an ABI PRISM® 7500 Sequence Detection System (Applied

Biosystems™). DNA was amplified using the GlobalFiler™ PCR kit on a ProFlex™ Dual 96-Well PCR System (Thermo Fisher Scientific). Cycling conditions were as per manufacturer's recommendations for 29 cycles, with 400 pg of DNA or a maximum DNA volume of 15 µL if 400 pg was not available, in a final volume of 25 µL. Amplified DNA fragments were resolved using an ABI 3500xl Genetic Analyser (Applied Biosystems™).

3.3.3.3 DNA profile analysis and comparison

Electropherograms were generated from all 147 shelf samples and were analysed using GeneMapper® ID-X Software V1.6 (Thermo Fisher Scientific) with an allele analytical threshold (AT) of 50 relative fluorescence units (RFU). No alleles were present within 23 of the 147 electropherograms analysed. For each DNA profile generated (N=124), the minimum number of individuals required to reasonably explain the profile was determined using peak heights and a minimum allele count. STRmix™ V2.7 was used to deconvolute DNA profiles into individual contributor profiles using in-house derived settings. DNA profiles which contained alleles from 1-4 contributors (N = 119) were analysed in STRmix™. Due to limitations in computing power, profiles which contained more than four contributors (N = 5) were analysed in STRmix™ V2.8 using a top-down DNA profile analysis [57, 58]. This analysis method allowed the major profile contributors to be captured and maximised the amount of useful information, which could be obtained.

Profiles generated from the shelf samples were compared to de-identified DNA profiles on the FSSA staff elimination database (N = 1801). This database consists of DNA profiles of all individuals who work within FSSA, including students and laboratory visitors, and individuals who may attend crime scenes or be involved in the handling of exhibits. Of the 1801 individuals on the database, approximately 120 are FSSA employees and 81 provided informed consent to be identified from their DNA profile within this study. Ethics approval was obtained from the Research and Development Committee of Forensic Science SA and the Social and Behavioural Research Ethics Committee of Flinders University.

For each comparison, a likelihood ratio (LR) was calculated using the opposing H1 and H2 propositions:

H1) the staff member and (N-1) unrelated individuals are the sources of DNA

H2) N unknown individuals, unrelated to the staff member are the sources of DNA

(where N is the number of profile contributors). An account for co-ancestry was not applied to the LR calculations and the point estimate value was used. Australian Caucasian allele frequency data was used for LR calculations [59]. A minimum LR cut-off of 10,000 was used to minimise adventitious matches, as per standard FSSA protocol.

The mixture-to-mixture comparison function of STRmix™ was used to compare DNA profiles generated from all shelf samples, which contained 1-4 contributors (N = 119). The five profiles

analysed using a top-down approach were not included in the mixture-to-mixture comparisons. For each mixture-to-mixture comparison, a LR was produced using the propositions:

H1) the two mixtures share a common DNA donor or

H2) there are no common DNA donors within the two mixtures.

Again, a minimum LR threshold of 10,000 was used in mixture-to-mixture comparisons.

Mixture-to-mixture analysis was also used to compare DNA profiles generated from shelf samples to the profiles generated from exhibits stored on the shelves sampled throughout the duration of the study as part of routine casework examinations. The numbers of exhibits stored on each shelf sampled and the numbers of exhibit profiles compared to samples in mixture-to-mixture analyses are displayed in Table 3.1.

Table 3.1. Number of exhibits stored on each shelf sampled throughout the 14 weeks duration of the study and number of profiles obtained from the exhibits that were used in mixture-to-mixture profile analysis.

Compactus unit	Shelf	Number of exhibits on shelf	Number of exhibit profiles compared to samples
1	Top	44	31
	Middle	93	64
	Bottom	33	28
2	Top	39	32
	Middle	28	15
	Bottom	34	17
3	Top	27	17
	Middle	25	14
	Bottom	14	7
6	Top	42	16
	Middle	5	6
	Bottom	9	8
7	Top	23	1
	Middle	14	2
	Bottom	15	0
Total		445	258

There was a total of 445 exhibits stored on all shelves sampled throughout the sampling period and 258 exhibit profiles were compared to shelf samples. DNA profiles were not generated from some exhibits, due to low DNA quantities within samples taken from the item. There were no DNA profiles available for some exhibits, as they were examined by a department other than biology and therefore not submitted for biological examination.

Exhibit profiles that were previously determined to contain more than four contributors using an AT of 50 RFU were re-analysed in GeneMapper® ID-X using an allele detection limit of 250 RFU (N = 42). This method is not carried out in casework at FSSA but was used within this study to maximise the amount of useful information which could be obtained from the exhibit profiles. Due to their complexity, 11 of the reanalysed exhibit profiles could not be further used.

3.3.3.4 Statistical analysis

Logistic Regression analysis was performed to determine if time was a significant factor in the probability of detecting DNA. Logistic Regression analysis was also performed to determine if compactus status (whether the compactus unit was full of exhibits or empty for the duration of the study) and shelf height were significant factors in the probability of detecting DNA, when considered together with time. For samples where DNA was detected, linear regression analysis was performed to determine whether there was a relationship between sampling time interval and shelf height and the amount of DNA (log10 ng of DNA).

Linear Regression analysis was also performed to determine whether there was a relationship between sampling time interval, shelf height and compactus status and the number of profile contributors from samples. All analyses were performed using R Software V4.1.2 and R Studio V2022.02.0 [60, 61] and a significance level of $p< 0.05$ was used for each analysis.

3.3.4 Results

3.3.4.1 DNA yield and profile contributors

Shelves within the FSSA exhibit storeroom were sampled to investigate the amount of DNA accumulating over time. The quantities of DNA (ng) recovered from the 21 shelf swabs taken (three swabs taken from each compactus unit) at seven time points are displayed in Figure 3.2. The highest DNA quantity detected within the study was 14.92 ng, which was obtained from the bottom shelf of compactus unit 2, after 8 weeks. An undetectable DNA quantity was obtained from 43 of the 147 samples taken. When considering the probability of detecting DNA within a sample, compactus status ($p= 0.008$) and time ($p= 2.1 \times 10^{-6}$) were both found to be significant. There was an increased probability of detecting DNA for compactus units which contained exhibits and the probability of detecting DNA increased with time. Shelf height did not have a significant effect on the probability of detecting DNA ($p=0.91$).

For samples which yielded a detectable DNA quantity, a statistically significant relationship was identified between the time interval and the total amount of DNA recovered from shelves, ($p =6.7 \times 10^{-6}$). In general, as time increased, the DNA quantity also increased. For samples which yielded a detectable DNA quantity, compactus status ($p= 0.20$) and shelf height ($p = 0.10$) did not have a significant effect on the quantity of DNA accumulating on shelves.

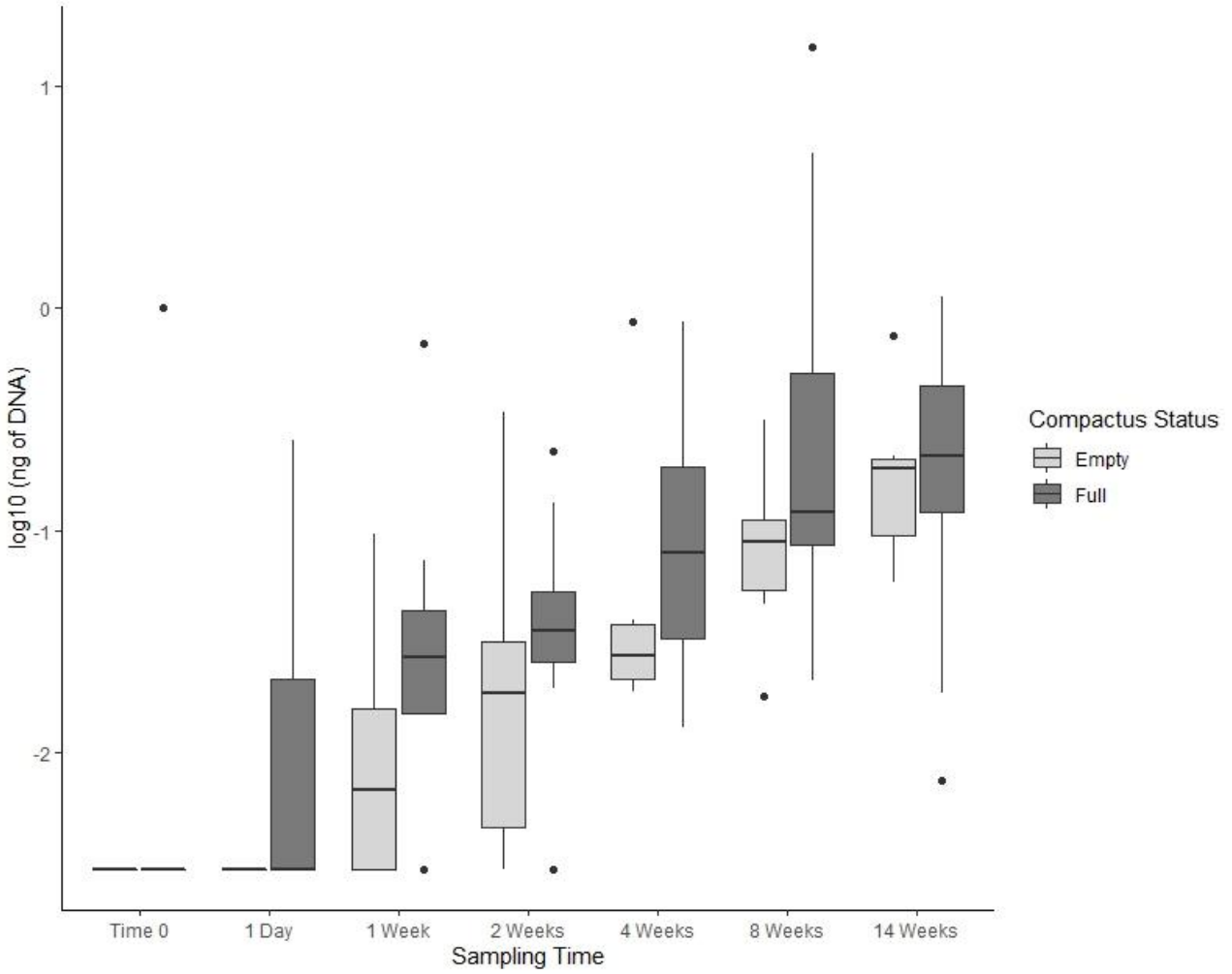

Figure 3.2. The log10 DNA quantities (ng) recovered from shelf samples taken at each time point. Samples which yielded an undetected DNA quantity were given a value of 0.00005 ng (-4.3 on a log scale), which is half of the limit of detection of the Quantifiler™ Trio DNA Quantification Kit. A top, middle, and bottom shelf within two of the seven compactus units sampled were kept empty for the duration of the study, therefore six of the 21 shelves sampled at each time point were within compactus units that did not contain exhibits (empty) and the remaining 15 shelves were within compactus units containing exhibits (full).

Electropherograms were generated from all 147 samples and contained alleles ranging from 0 to 8 contributors. Alleles from at least one contributor were present within 124 of the 147 electropherograms generated. The number of contributors observed within profiles are displayed in Figure 3.3.

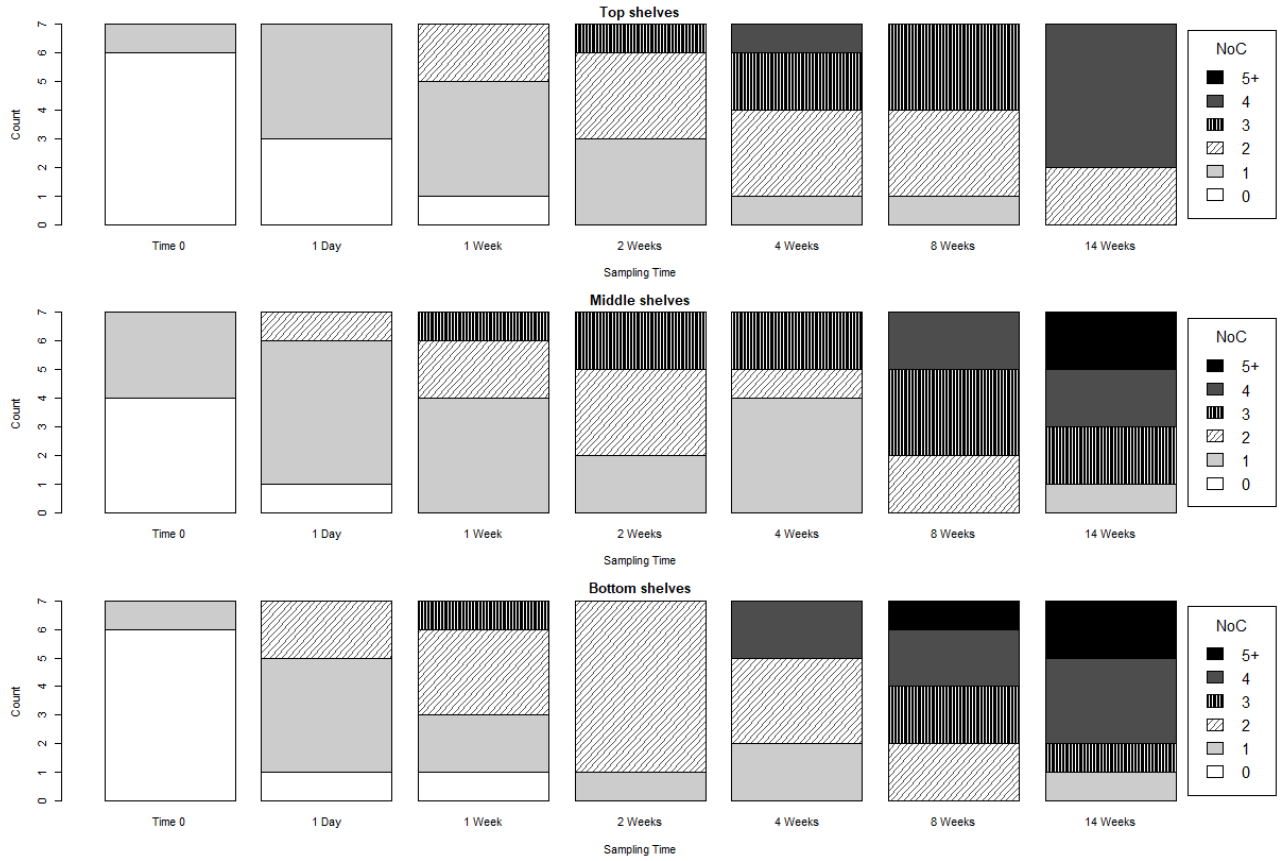

Figure 3.3. Numbers of contributors (NoC) to profiles generated from samples taken at each time point. NoC were assessed using a peak detection threshold of 50 RFU.

As time increased, there was a significant increase in the number of DNA profile contributors observed ($p < 2.0\times10^{-16}$). The compactus status did not influence the number of profile contributors accumulating on shelves ($p = 0.13$). A relationship was observed between shelf height and the number of profile contributors ($p = 0.05$). Generally, a higher number of profile contributors were present in samples taken from lower shelves.

3.3.4.2 Staff database matches

To identify the source of DNA accumulating on exhibit storeroom shelves, DNA profiles generated from samples were compared to the DNA profiles on the FSSA elimination database. At least one staff member was matched to 31 % of the samples taken from storeroom shelves. The comparison between the shelf samples and elimination database yielded a total of 55 LRs > 10 000, which consisted of matches to 35 different individuals, from 7 departments. A visual representation of the location of samples which yielded staff matches and the individuals' departments has been displayed in Figure 3.4. The strength of the LR for each staff match is displayed in Figure 3.5, with the match proportions of individuals from each workgroup who yielded an LR indicated in Figure 3.6.

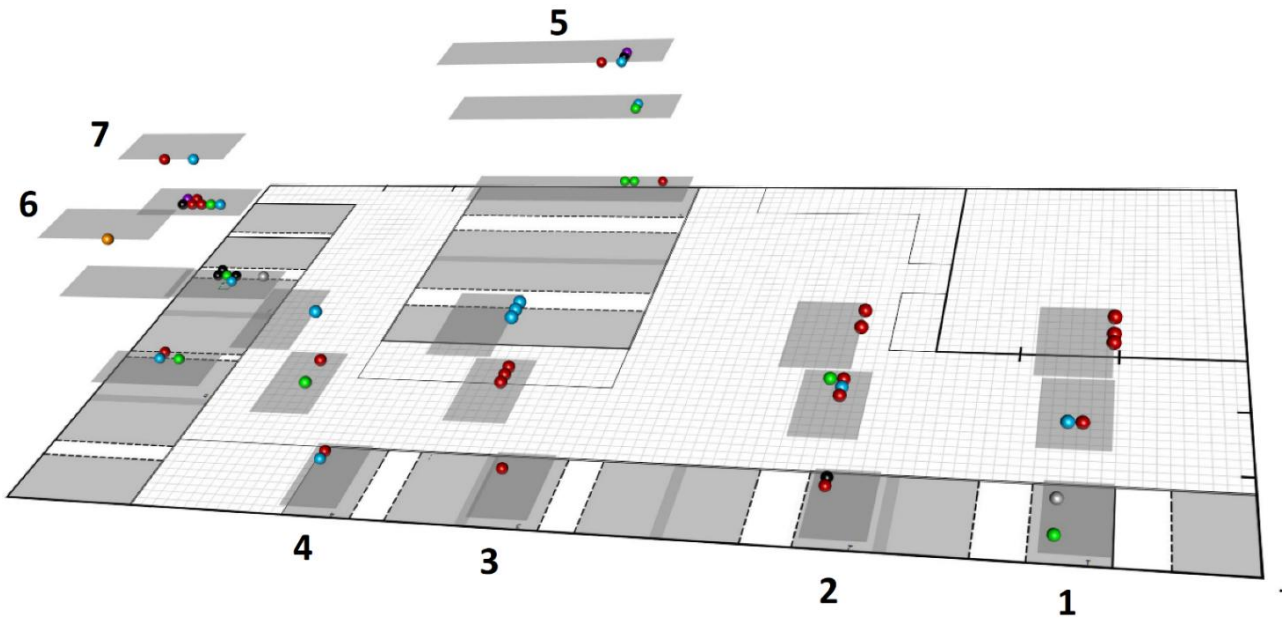

Figure 3.4. Three-dimensional (3D) modelled FSSA exhibit storeroom floorplan. The top, middle and bottom shelf for each compactus unit sampled has been represented using a rectangular area shaded in grey. Each unit sampled has been numbered in bold text, as per Figure 1. Spheres of different colours represent staff members from different departments, who generated an LR to any shelf samples (blue = Biology, red = Administration, green = Chemistry, black = Police, orange = Executive, purple = Pathology and light grey = Toxicology).

There were no matches generated between the FSSA elimination database and samples taken from shelves directly after cleaning (time 0). Samples taken at 8 weeks yielded the highest number of staff matches (15), followed by samples taken at 14 weeks (14). Staff DNA was detected on the bottom shelf of two different compactus units (1 & 7), only one day after cleaning the shelves.

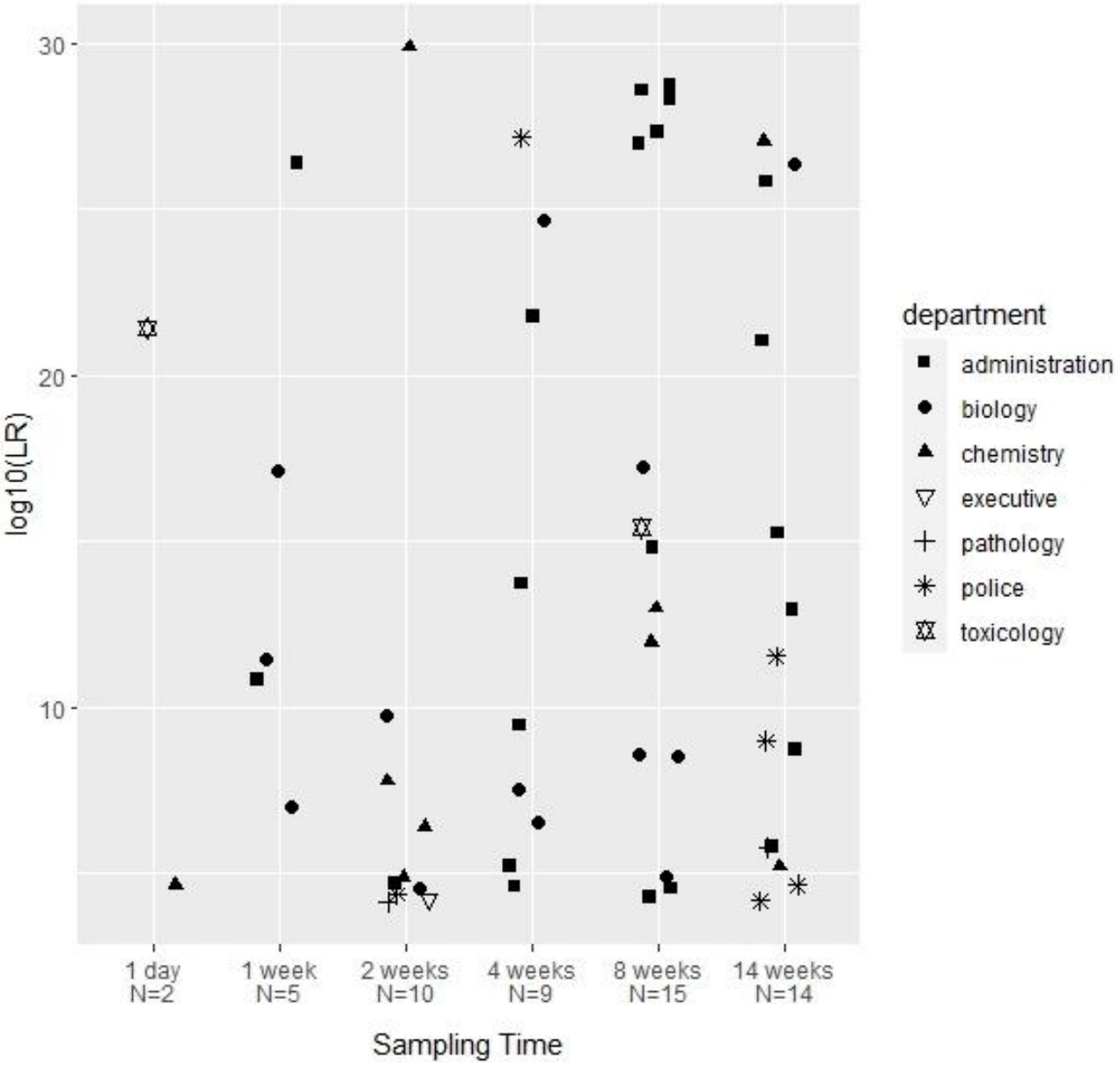

Figure 3.5. log10 LR of staff matches yielded at different time points. Different shapes indicate the different departments of individuals who yielded an LR. Time 0 has been emitted from the figure as there were no staff matches yielded to samples taken at this time point.

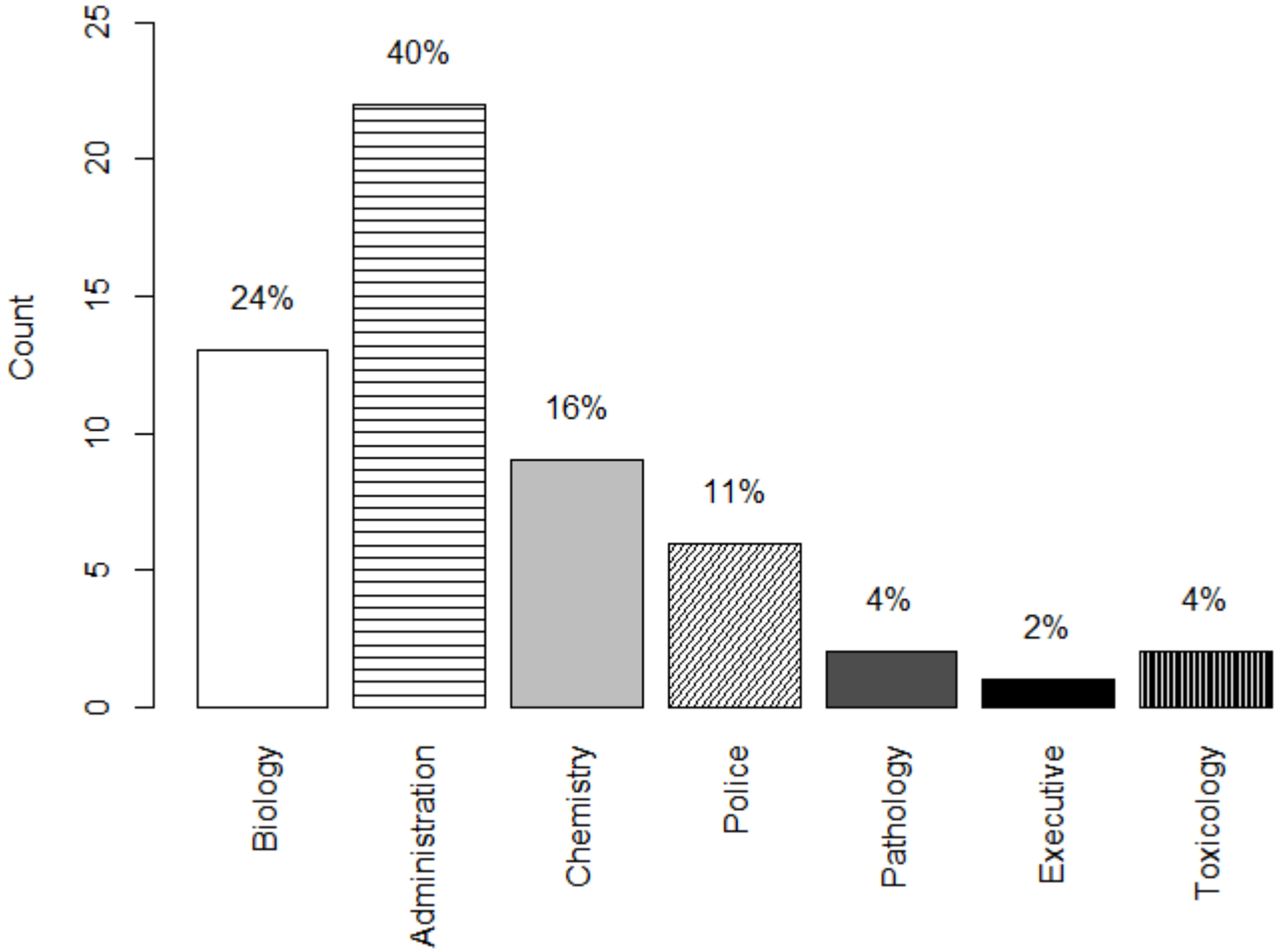

Figure 3.6. Proportions of individuals from each department matched to the samples taken from storeroom shelves.

Individuals who work within the FSSA Administration department were detected on shelves most frequently, compared to individuals from all other departments. Some individuals were detected on shelves more frequently than others. For example, an LR was generated between eight samples (taken from five different shelves, within four compactus units) and one individual from Administration, which was the higest number of matches observed to a single individual. A different individual from the same department was matched to four samples.

3.3.4.3 Mixture to mixture comparisons between shelf samples

To identify DNA from common contributors which accumulated on exhibit storeroom shelves and not able to be attributed to staff members whose DNA is on the elimination database, profiles generated from shelf samples were compared to each other. There were 44 common profile contributors identified between different shelf samples. There were 22 instances where the common contributor was the major contributor to both profiles and 6 instances where the common contributor was not the major contributor to either profile. Seven contributors were only detected within samples taken from the same compactus unit, and two of these contributors were only

detected in samples taken from the same shelf. The remaining 37 contributors were identified within samples taken from at least two different compactus units.

Common elimination database matches between samples accounted for 31 of the 44 total common contributors identified. Samples identified to contain common staff contributors have been displayed in Figure 3.11, with a visual representation of the locations of samples identified to contain common contributors displayed in Figure 3.12. The remaining 13 common contributors could not be attributed to staff members whose DNA is on the elimination database. Samples identified to contain a common non-staff DNA profile contributor are displayed in Figure 3.7, with a visual representation of the locations of the samples identified to contain common contributors displayed in Figure 3.13.

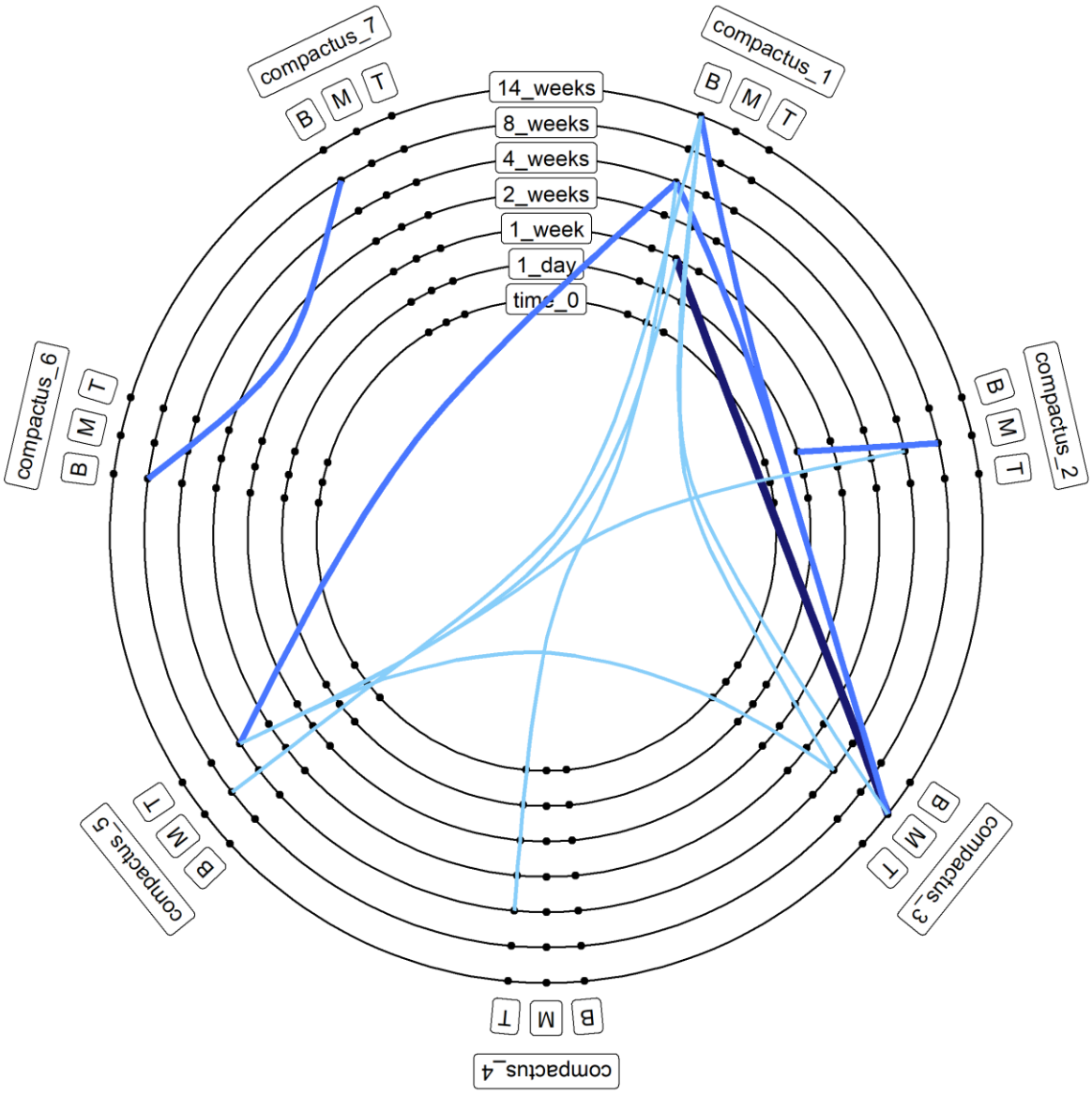

Figure 3.7. Samples taken from the top (T), middle (M) and bottom (B) shelf of each compactus unit have been represented using dots. The rings of the circle represent different time points samples

were taken. Thick coloured lines connecting samples represent common profile contributors, which were identified through mixture-to-mixture analysis and were not able to be attributed to staff whose DNA is on the elimination database. The colour of the line indicates the strength of the LR for each match (the stronger the LR, the darker the colour of the line).

3.3.4.4 Mixture to mixture comparisons between shelf samples and exhibit profiles

To determine whether DNA from exhibits is accumulating on storeroom shelves, profiles generated from shelf samples were compared to profiles generated from exhibits stored within the units sampled over the 14-week period. A total of 258 exhibit profiles were compared to the shelf samples, resulting in 32,072 comparisons being performed. A common profile contributor was identified between a shelf sample and profile generated from a casework exhibit in three of these comparisons performed. The storage location of the exhibit, location of the matched sample and log10 LR yielded for each match have been displayed in Figure 3.8.

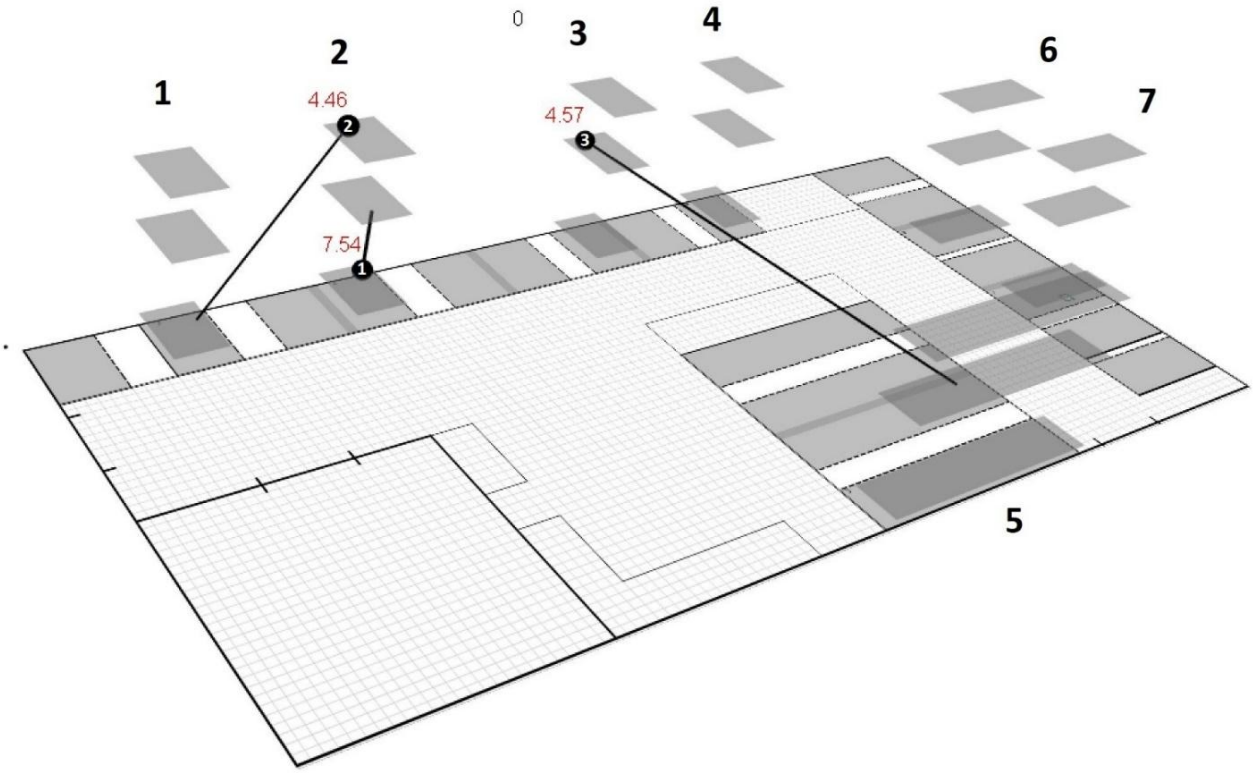

Figure 3.8. FSSA exhibit storeroom floorplan modelled in 3D. Each compactus unit within the storeroom has been represented using a rectangular area shaded in grey. Each unit sampled has been numbered in bold text as per Figure 1 and the top, middle and bottom shelf for each of these units are displayed as grey rectangles in a 3D configuration. Spheres represent the storage location of exhibits, which were stored on shelves sampled at some point over the sampling duration and yielded an LR >10,000 in mixture to mixture analyses performed between shelf samples and a sample taken from an exhibit. Each exhibit has been allocated a number, which is displayed in white text within each sphere, to allow them to differentiated. The end of the line connected to each sphere

represents the origin of the shelf sample which yielded an LR for a common contributor with the exhibit. The log10 LR for each contributor match is indicated in red text next to each sphere.

The common contributor identified in the sample taken from the middle shelf of compactus two at 4 weeks and the profile of the exhibit that was stored on the bottom shelf of compactus two (exhibit 1, as per Figure 3.8) was determined to be an FSSA staff member, through comparison with the staff elimination database. Exhibit movement and storage details for the two other exhibits (exhibits 2 & 3, as per Figure 3.8) which yielded an LR in the mixture-to-mixture comparisons to shelf samples are displayed in Figure 3.9 and Figure 3.10.

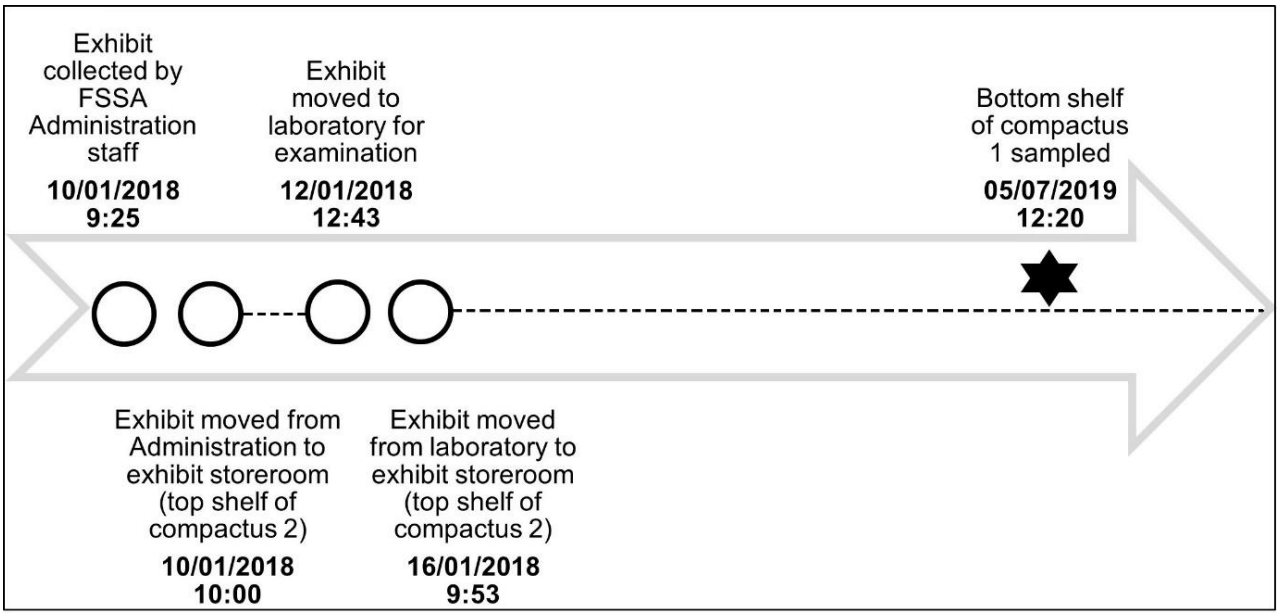

Figure 3.9. Movement timeline of exhibit 2, which was identified to share a common profile contributor with a sample taken from the bottom shelf of compactus 1, after 1 week. Circles represent each exhibit movement event, and the dashed line indicates the time periods where the exhibit was stored on the top shelf of compactus 2 within the exhibit storeroom. The star indicates when the sample, which yielded a match to an exhibit profile contributor, was taken from the storeroom shelf.

The sample which was identified to share a common profile contributor with exhibit 2, was not taken from the shelf where the exhibit was stored. This sample was taken from the shelf after exhibit 2 had been stored within the storeroom for approximately 537 days. There is one compactus unit located between the shelf where the exhibit was stored (top shelf of compactus 2) and the shelf where the sample which yielded the common profile contributor was taken (bottom shelf of compactus 1).

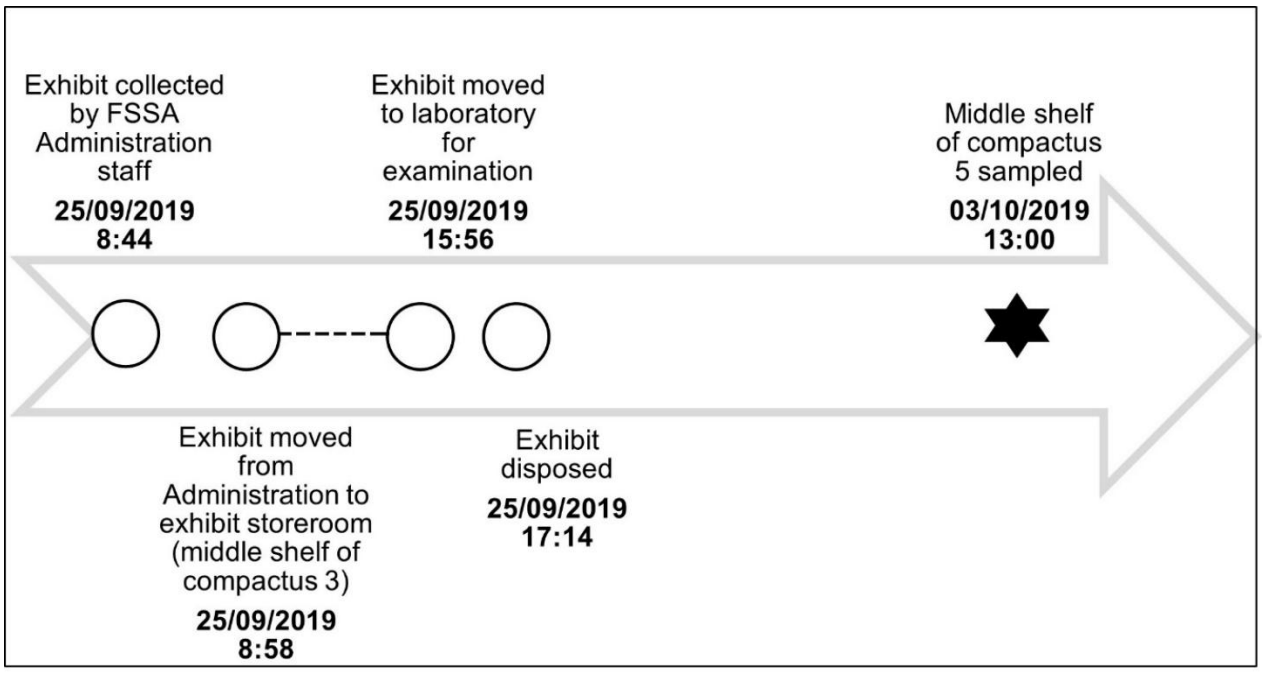

Figure 3.10. Movement timeline of exhibit 3, which was identified to share a common profile contributor with a sample taken from the middle shelf of compactus 5, after 14 weeks. Circles represent each exhibit movement event, and the dashed line indicates the period where the exhibit was stored on the middle shelf of compactus 3 within the exhibit storeroom. The star indicates when the sample, which yielded a match to an exhibit profile contributor, was taken.

Exhibit 3 was stored within the exhibit storeroom for an approximate total of 7 hours, before being moved to the laboratory for examination and then disposed of. The shelf sample which yielded a match to this exhibit was taken approximately 8 days after the exhibit was last present within the storeroom. The sample which was identified to share a common profile contributor with the exhibit profile was taken from a compactus unit which did not contain any exhibits for the duration of the study and was therefore not the shelf where the exhibit was stored.

3.3.5 Discussion

Research which investigates DNA accumulation within forensic workspaces provides valuable insight into the transfer and persistence of DNA within operational forensic laboratories. To evaluate the risk of moving to more sensitive DNA profiling technologies and assess the adequacy of current exhibit handling and storage procedures, a greater understanding of DNA accumulation and transfer within areas where exhibits are handled and stored is required. This study aimed to investigate DNA accumulation within forensic exhibit storage locations and explore the DNA transfer which occurs between exhibit packaging and forensic workspaces. Such information provides insight into the mechanisms which may be involved in the incidental contamination of exhibits, as a result of exhibit handling and throughout the forensic process.

Within this study, the ability for DNA to accumulate on surfaces within a forensic exhibit storeroom was studied over a 14-week period. Undetected DNA quantities yielded from 20 of the 21 samples taken immediately after cleaning suggest that the cleaning process was effective at removing much of the DNA from the shelves at the beginning of the study. As the whole shelf was not sampled after cleaning, it is possible but not known whether DNA persisted on the rest of the surface after cleaning. The many samples which yielded an undetectable DNA quantity indicate that the levels of DNA which may have persisted on the shelf after cleaning were not likely to be high enough to have significantly impacted the DNA quantities observed within this study. The presence of this DNA may have caused a slight increase in the number of profile contributors observed within samples. There were 1-2 alleles present within the DNA profiles generated from nine of the 21 samples taken immediately after cleaning. If such levels of DNA persisted on the whole surface of the shelves after cleaning, it is expected that the results of this study would not have been significantly impacted, as any matches which may have been generated in staff comparisons or mixture to mixture analyses would likely not have yielded a LR above the detection threshold and therefore would not have been observed.

The probability of detecting DNA within a sample and the DNA amount detected was observed to increase with time. The numbers of profile contributors observed within samples also increased with time, which indicates that the increase in DNA quantity was attributable to multiple individuals. These results indicate that DNA accumulates on exhibit storeroom shelves over time and are consistent with other studies which identify time as being a significant factor in the accumulation of DNA [45]. It is expected with increasing time that there are more opportunities for events which may result in the transfer of DNA to occur and therefore an increase in the amount of DNA which accumulates. It is not surprising that DNA accumulates on exhibit storeroom shelves, as these surfaces are not regularly cleaned, and frequently make contact with individuals and items that are not free from DNA. Without the requirement for staff who enter the storeroom to wear PPE, the opportunity for individuals to deposit DNA within their surroundings through direct contact with a surface [3, 4] or through speaking coughing or sneezing [32, 48, 49], readily exists. Observations within this study are consistent with research that demonstrates the ability for an individual's DNA to accumulate on surfaces within areas they frequent [29, 45, 46], even when direct contact with a surface has not been made [45].

There was a higher probability of detecting DNA on shelves within compactus units which contained exhibits, compared to compactus units which did not. Generally, higher amounts of DNA were observed to accumulate on shelves within compactus units that contained exhibits for the duration of the study. Within these compactus units, it is expected that there was more opportunity for DNA transfer to occur through contact with the outside of exhibit bags stored on the shelves and staff who reach in and out of these units to access the exhibits stored within. The shelves within full compactus units were constantly in direct contact with exhibit packaging throughout the

sampling duration. As compactus units are moved, friction between items within the unit would occur, which may increase the chance of DNA transfer between shelves and the packaging of exhibits. Higher amounts of DNA are generally transferred in contacts which involve friction and pressure [10, 62]. While it is expected that some level of bi-directional DNA transfer occurs with all contacts made between substrates [63, 64], there is limited data that details the extent of DNA transfer from the surface of an exhibit bag to other substrates. The instances of frequent direct contacts within compactus units containing exhibits could account for the difference in the DNA quantities observed between the empty and full compactus units. As DNA was still observed to accumulate on shelves which did not contain exhibits, DNA transferred from exhibit bags does not account for all of the accumulating DNA.

The accumulation of DNA in compactus units which did not contain exhibits suggests that some level of aerial DNA transfer is occurring within the environment. It is possible that DNA may be circulating throughout the storeroom through the air conditioning system, but the extent of this transfer is unknown. If the assumption is made that no individuals contacted shelves, or placed items onto the shelves, within the empty compactus units, the DNA could not have been deposited through direct contact. Individuals who entered the storeroom may have deposited their DNA onto shelves through speaking [48], however, this mechanism does not explain how DNA from a police employee, who does not enter the storeroom, was detected on a shelf within an empty compactus unit. To better understand the ability for DNA to be transferred aerially, and persist in the air within an environment, further research is required.

Compactus status did not have an impact on the number of profile contributors observed within samples. There was no correlation between the number of exhibits on a shelf and the number of profile contributors observed. While similar quantities of DNA were observed to accumulate on the top, middle and bottom shelves at each time point, higher numbers of profile contributors were generally observed in samples taken from shelves with a lower height. This could be explained by aerial DNA and cells which have been shed from clothing, skin and other items falling on to lower surfaces due to gravity.

This study demonstrates the propensity for DNA from forensic and police employees to accumulate on shelves within forensic storage locations. Staff DNA was detected on shelves only one day after cleaning and samples taken at 8 and 14 weeks generated more matches to staff, compared to other time points. The proportions of individuals matched from each FSSA department correspond with how often individuals within the department frequent the storeroom. DNA from individuals who work within the FSSA Administration department was detected more than individuals from other departments. This result can be explained by the fact that Administration staff frequent the exhibit storeroom and regularly make contact with the exhibit bags and workspaces within the storeroom as part of their routine duties. It is therefore likely that Administration employees have more

opportunity to deposit DNA within the storeroom, compared to individuals from other departments. Individuals from Biology and Chemistry, the second and third most commonly detected departments, frequently access the exhibit storeroom to collect exhibits for examination and return exhibits to the storeroom after examination. Less matches were generated to Executive staff, compared to other departments. These individuals do not frequently enter the exhibit storeroom, but their working department is located on the same floor as the exhibit storeroom. It is possible that DNA from the Executive staff member was deposited somewhere throughout the floor and indirectly transferred into the storeroom by another individual.

The match proportions of Police employees do not correspond with how often these individuals frequent the storeroom. Police employees do not enter the FSSA exhibit storeroom, therefore the presence of DNA from these individuals can only be explained through indirect transfer mechanisms. One mechanism which may explain the detection of police DNA within the storeroom is where DNA has been directly deposited onto the evidence bag [56] or to Administration staff at the time of logging in exhibits, and then transferred onto the storeroom shelf.

Thirty-eight percent of the DNA profiles analysed within this study generated at least one match to the staff database or an exhibit profile. Therefore, much of the DNA detected within the storeroom did not generate any matches to the elimination database or exhibit profiles. Two of the 13 common contributors were only observed once. A single DNA donor appeared to account for the remaining 11 common contributors identified. This contributor was identified within nine samples which were taken from five different compactus units at four different time points. DNA that had originated from another individual and was present on staff who work within the exhibit storeroom (non-self DNA), could account for some of the DNA which was detected and not attributed to staff [64-66]. Individuals who have handled exhibit bags prior to being transported to FSSA, and are not on the elimination database, could also account for some of the common profile contributors and DNA which was detected. Not all police employees are on the FSSA elimination database, which means if DNA from any of these individuals was present, a match would not have been detected.

There were three instances where a common DNA profile contributor was observed between a shelf sample and an exhibit profile. One of these instances could be explained by DNA from a staff member being detected within the profile generated from an exhibit (exhibit 1) and on a shelf sample. Unlike for exhibit 1, there was no clear explanation for the remaining two instances where a common DNA profile contributor was observed between a shelf sample and an exhibit profile. The common contributor identified in the profile from exhibit 2 could not be assigned to any person in the case, whereas the contributor in the profile from exhibit 3 was identified as the complainant. Exhibits are enclosed within a sealed evidence bag within the storeroom, therefore the detection of exhibit DNA on a shelf could only be the result of an indirect DNA transfer mechanism. Exhibit DNA which was transferred to the exterior of the evidence packaging and then transferred to the

shelf through another intermediary surface may explain the common contributor identified between exhibit 2 and the storeroom shelf sample. DNA transfer that occurs through direct contact between exhibit packages from different shelves, while multiple items are collected and transported to or from the laboratory, is an example of one event which may facilitate such transfer. For this mechanism to occur, DNA from exhibit 2 would have needed to persist on a surface within the storeroom for 535 days. The exhibit storeroom is a temperature controlled environment, which is protected from UV exposure, and therefore would likely provide ideal conditions for DNA persistence.

DNA transferred from the exhibit to the bag, during examination, does not explain the common contributor identified between exhibit 3 and the shelf, as the exhibit bag was not returned to the storeroom after examination. If DNA (perhaps in cellular form) from this exhibit was transferred to the exterior of the exhibit packaging during the initial packaging process, as previously demonstrated in [55], it may be possible that DNA was dislodged from the packaging and transferred to another item, or area, within the storeroom. The shelf where the common contributor was detected did not contain any other exhibit bags, or items that may have facilitated this transfer, which suggests that the DNA was transferred via the air within the environment (such as described in [67]). The prevalence of these transfer mechanisms is unknown and research which involves aerial DNA transfer would assist with better understanding the ability for these mechanisms to occur.

It is also possible that the common contributors observed between the profiles of exhibits 2 and 3, and the samples taken from the shelves are the result of an adventitious match. The LR generated between the common contributors identified between shelf samples and exhibits 2 and 3 were considerably smaller than the LR generated to exhibit 1, which can be explained by the fact that the DNA profiles generated from the samples which yielded matches to exhibits 2 and 3 contained fewer alleles. Within this study there were a total number of 32,072 comparisons performed between profiles generated from shelf samples and exhibits. Increasing the number of comparisons also subsequently increases the probability of generating a match to a sample by chance. With the lack of research that investigates the ability for DNA to be transferred from exhibit packaging to other substrates, and the prevalence of aerial DNA transfer, it cannot be determined which explanation best explains the common contributors observed between the shelf samples and the profiles generated from exhibits 2 and 3.

The detection of exhibit DNA on shelves is of concern, as it demonstrates the potential for exhibit DNA, which has been transferred to its package, to be further transferred to other surfaces. The ability for DNA to be transferred between the packaging of exhibits, or from storage shelf surfaces to exhibit packaging has not been extensively studied. If the ability exists for exhibit DNA to be transferred from an exhibit to a storeroom shelf or an exhibit bag, then to the package of a second

exhibit and then onto the second exhibit itself, there is the potential for false inclusions between exhibits, and cases, to occur.

It has previously been suggested that increased levels of DNA on the exterior of exhibit packaging presents an increased risk of exhibit contamination, through DNA transferred from the packaging to the exhibit during the examination process [31]. Within one study DNA was observed to accumulate on the packaging of exhibits which were within an exhibit storeroom, however it was not known whether the accumulating DNA was transferred from staff, the shelf surface or from other exhibit packaging [55]. While the exact transfer mechanisms are unknown, the findings within this study reinforce that DNA accumulating on storeroom shelves is likely to be a source of the DNA which has been previously observed to accumulate on the exterior of exhibit bags [55, 56]. Changing gloves between handling exhibits and exhibit packaging is necessary to minimise the risk of transferring such accumulating DNA to the exhibit. Implementing procedures which reduce the amount of DNA accumulating within exhibit storerooms is likely to assist with reducing the amount of DNA accumulating on exhibit bags and would therefore minimise the risk of exhibit contamination. Frequent cleaning of surfaces within the exhibit storeroom would reduce the amount of DNA accumulating on shelves. Wearing PPE while entering the exhibit storeroom and handling exhibits would also assist to reduce the amount of DNA accumulating. Additionally, the use of PPE within the storeroom would reduce the accumulation of non-self DNA from individuals who work within the environment. Ongoing environmental sampling could be used to monitor the levels of DNA accumulating within forensic exhibit storerooms.

The amount of DNA which was found to accumulate within the exhibit storeroom and could not be attributed to staff emphasises the importance of including all individuals who handle exhibits, or work within areas where exhibits are handled and stored, being present on a laboratory elimination database. Through the comparison of samples to a laboratory elimination database, instances where an exhibit is contaminated with staff DNA will generally be detected. In comparison, contamination by non-staff DNA, or DNA from another exhibit, is more likely to go undetected.

Within a previous study, where exhibit DNA was detected on the exterior of evidence bags after exhibit examination, the question of whether exhibit DNA was able to accumulate within exhibit storage locations was raised [56]. A common DNA profile contributor between a shelf sample and a profile generated from an exhibit, which could not be explained by the presence of staff DNA, was detected in 0.7 % of comparisons within this study. If the common contributors identified between exhibit profiles and shelf samples were the result of DNA being transferred between an exhibit and a storeroom shelf, there was a low prevalence of the detection of exhibit DNA within an exhibit storage location.

3.3.6 Conclusion

Exhibit storerooms are indoor, temperature controlled environments, which provide favourable conditions for DNA to accumulate and persist over extended durations of time. Frequent cleaning of surfaces within exhibit storage locations and wearing PPE while contacting exhibit bags and entering exhibit storerooms, may help to reduce the level of DNA accumulating within these environments. Procedures which minimise the amount of DNA accumulating within an exhibit storeroom minimise the accumulation of DNA on exhibit packaging and subsequently reduce the amount of DNA which could be transferred between an evidence bag and the exhibit within. We note that there are always additional measures that could be taken to further reduce contamination within a forensic laboratory. Once the most impactful measures have been taken then continuing to more extreme measures provide diminishing returns with respect to reducing contamination rate. There is a balance that must be struck between the cost/practicality of anti-contamination measures taken and the risk of contamination occurring. We have suggested a number of measures regarding exhibit packaging, storage and manipulation that would further reduce the contamination rate. Implementation of these measures is not mandatory, and we have shown the risk of contamination via these routes, while possible, is low. Whether a laboratory chooses to implement any or all of these measures would need to be decided based on a cost-benefit analysis.

In this study, it was observed that DNA from forensic staff, who enter the storeroom, and police staff, who do not enter the storeroom, could be detected within the environment. Much of the DNA accumulating within the storeroom had an unknown source, which reinforces the importance of including all individuals who handle exhibit bags, or work within exhibit storage locations on a laboratory elimination database. From this study, it is observed that there is a low prevalence of detecting exhibit DNA within an exhibit storeroom. These findings suggest that the ability for cross contamination between exhibits as a result of DNA accumulating within the forensic exhibit storeroom is minimal.

3.3.7 Acknowledgements

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3.3.8 References

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3.3.9 Supplementary Material

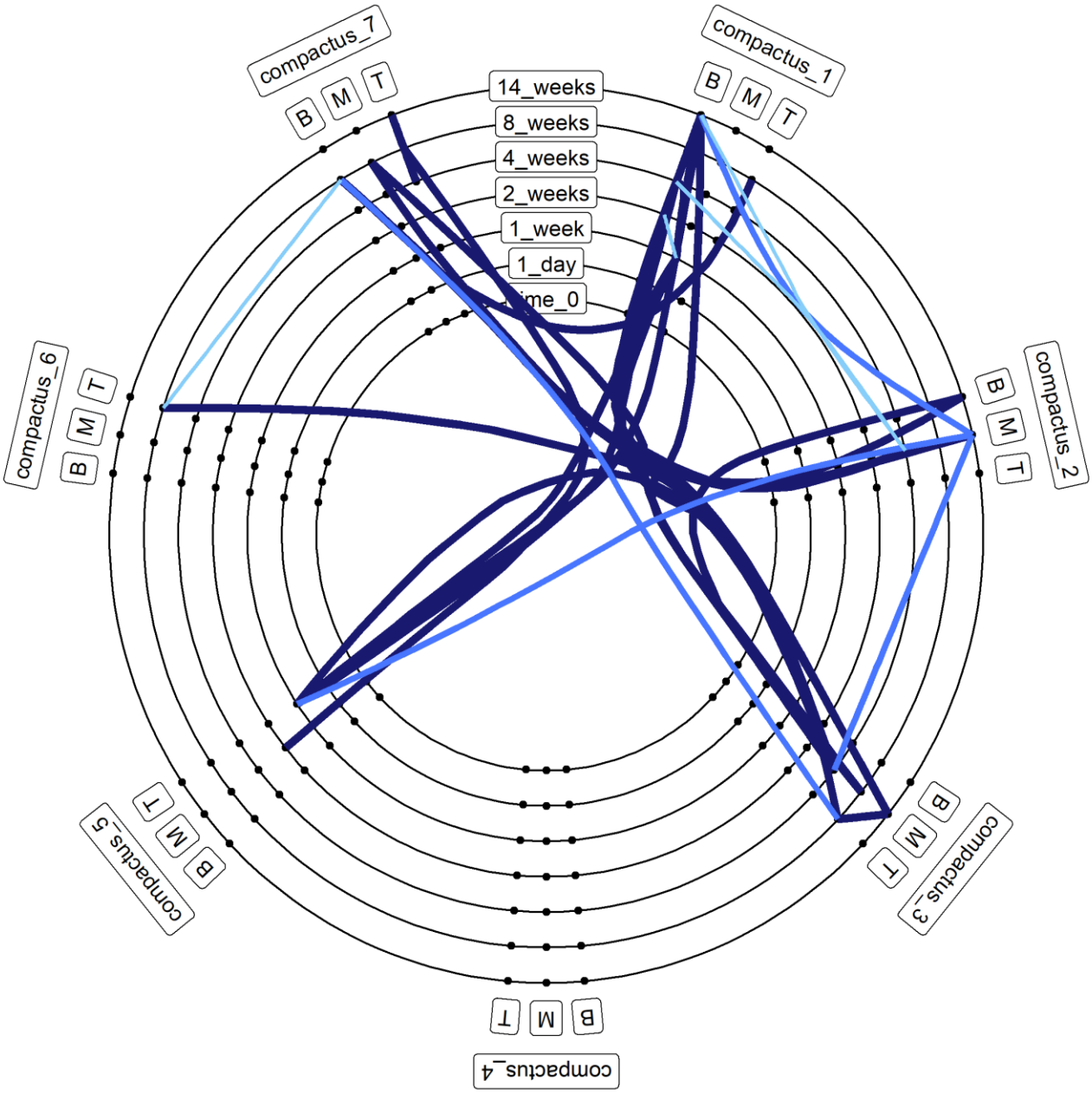

Figure 3.11. Samples taken from the top (T), middle (M) and bottom (B) shelf of each compactus unit have been represented using dots. The rings of the circle represent different time points samples were taken. Thick coloured lines connecting samples represent common profile contributors, which were identified through mixture-to-mixture analysis and were able to be attributed to staff whose DNA is on the elimination database. The colour of the line indicates the strength of the LR for each match (the stronger the LR, the darker the colour of the line).

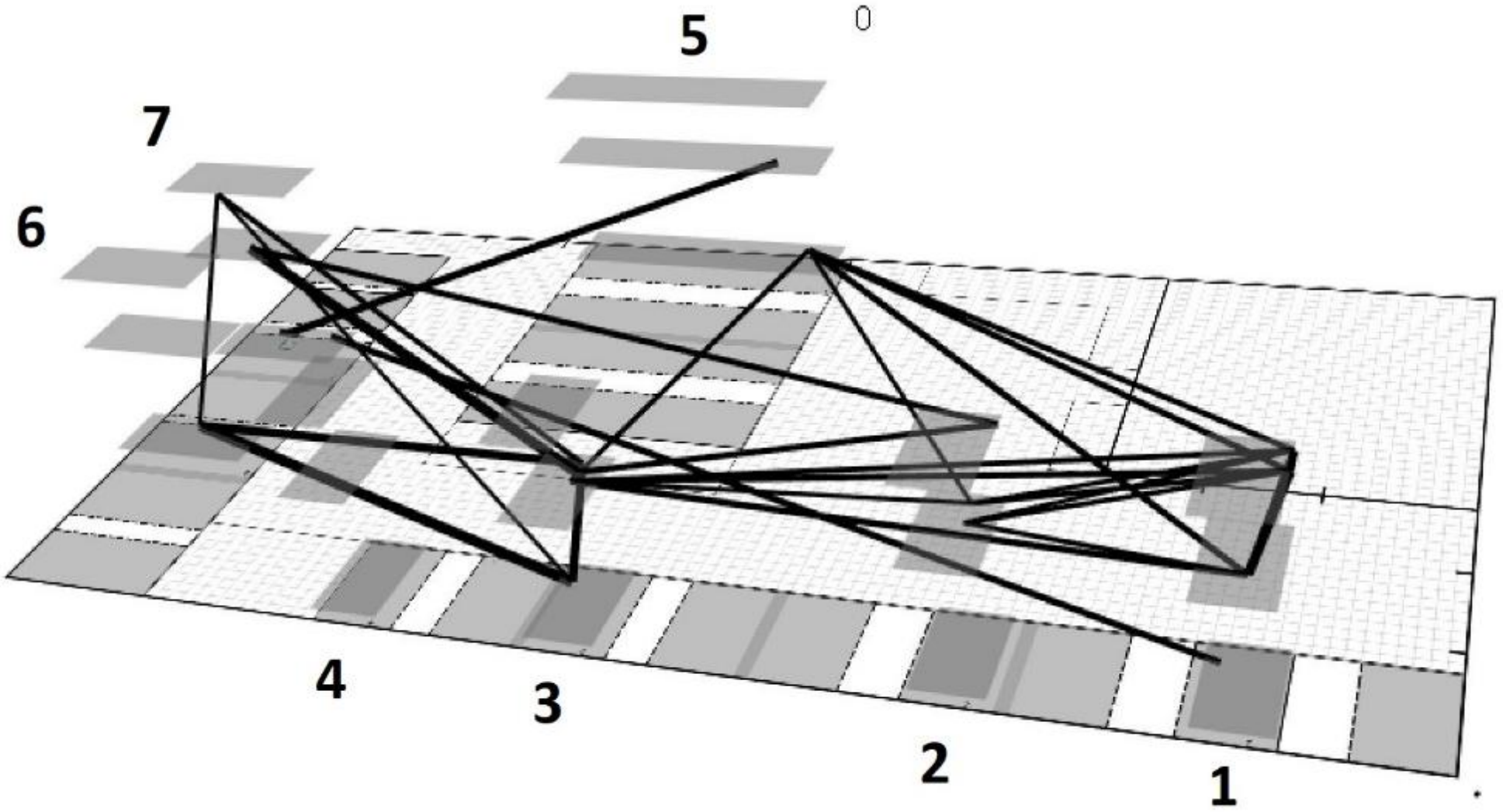

Figure 3.12. 3D floorplan of the FSSA exhibit storeroom with mixture to mixture DNA profile analysis results overlayed. Each compactus unit within the storeroom has been represented using a rectangular area shaded in grey. Each unit sampled has been numbered in bold text as per Figure 3.1 and the top, middle and bottom shelf for each of these units are displayed as grey rectangles in a 3D configuration. Lines have been used to connect shelves which share a common DNA profile contributor identified to be an individual on the laboratory elimination database with a LR > 10,000.

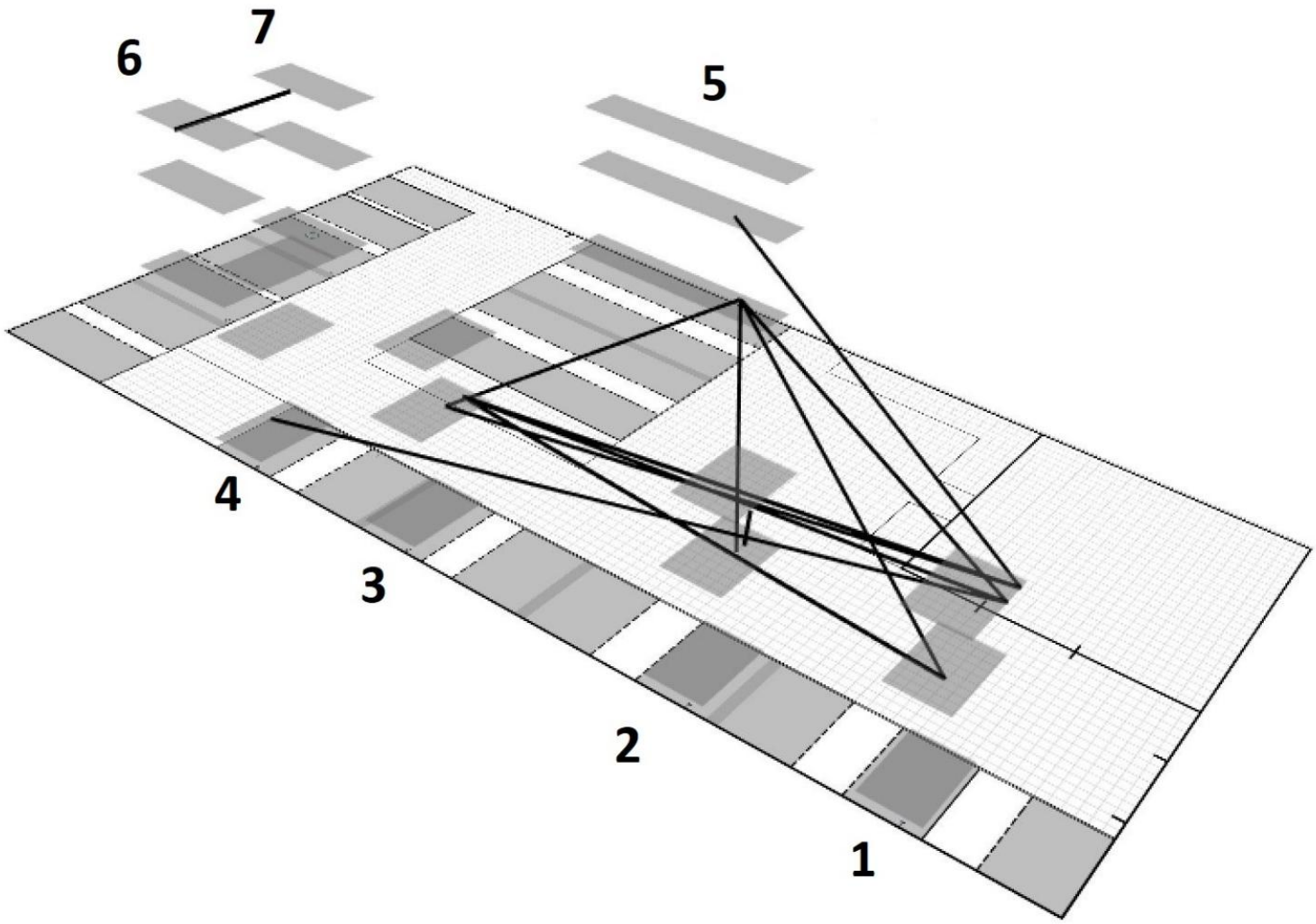

Figure 3.13. 3D floorplan of the FSSA exhibit storeroom with non-staff mixture to mixture DNA profile analysis results overlayed. Each compactus unit within the storeroom has been represented using a rectangular area shaded in grey. Each unit sampled has been numbered in bold text as per Figure 3.1 and the top, middle and bottom shelf for each of these units are displayed as grey rectangles in a 3D configuration. Lines have been used to connect shelves which share a common non-staff DNA profile contributor with a LR > 10,000.

CHAPTER 4 : DNA TRANSFER BETWEEN AND THROUGH EVIDENCE BAGS

4.1 Preface

The detection of DNA that could be attributed to exhibits emanating from the exterior of evidence bags (Chapter 2), and from surfaces within exhibit storage locations (Chapter 3), prompted the requirement to better understand the mechanisms involved in the accumulation and transfer of DNA from an exhibit. An additional question posed was whether DNA could migrate through the porous surface of paper evidence bags and subsequently transfer to the exterior of another paper bag; this is a concept that had not previously been studied. This chapter explores the potential for DNA to permeate the porous surface of a paper evidence bag and also to transfer between the surfaces of exhibit bag to a second surface. These concepts were first tested under highly controlled conditions to determine what transfer mechanisms are practically possible. Following this, transfer was tested within a semi-controlled environment, to determine what may be observed in more realistic exhibit handling scenarios. Results are reported in a manuscript draft, in a format ready to be submitted for publication.

4.2 Manuscript draft: The potential for DNA to transfer between and through the surface of paper evidence bags

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4.2.1 Abstract

Exhibit packaging is one essential stage in the collection and processing of all forensic evidence. While the brand and composition of evidence bags used by police, crime scene examiners and laboratories are likely to vary between jurisdictions, general packaging guidelines specify using paper evidence bags for exhibits containing biological material; this is to prevent microbial growth and sample degradation. Studies which explore the accumulation of DNA on the exterior of evidence bags and the potential for such DNA to be transferred to the exhibit, indicate that exhibit packaging is an inadvertent DNA transfer vector. It has previously been demonstrated that during the process of examination of an exhibit, DNA can be transferred between the exhibit packaging exterior and the exhibit within, even without direct contact. There are limited data regarding the mechanisms involved in such transfers, however possible explanations considered include: cells being dislodged from the exterior of the packaging and falling onto the exhibit; brief contact between the exhibit and the opening edges of the bag; and indirect transfer via a subsequent

contact with another intermediary substrate. One mechanism which has not been extensively considered is the potential for DNA to permeate the porous surface of paper evidence bags, with the effect of DNA migrating from the outside to the inside, or vice versa. Another mechanism which has not previously been investigated is the potential for DNA to transfer between the exterior surface of multiple evidence bags; this would be the case when multiple sealed packages come into direct contact during transport or storage. There are three related aims in this study: to examine the potential for DNA to permeate through the surface of a paper evidence bag and transfer between the exterior surface of paper evidence bags; the potential for DNA to transfer from a DNA bearing substrate, through the surface of an evidence bag and onto another substrate within a variety of conditions (time, pressure, friction, motion); and the potential for DNA to transfer between the surface of evidence bags using different contact types. This research is aimed at providing a better understanding of the mechanisms which may be responsible for contamination of exhibits by DNA and provides insight into whether current exhibit handling and storage procedures are still adequate given the highly sensitive DNA technologies currently being used.

4.2.2 Introduction

The packaging of an exhibit is a crucial stage in the collection and processing of all forensic evidence. Exhibit packaging assists with preserving the integrity of evidentiary items preventing the loss or gain of extraneous DNA resulting in the evidential item being compromised [1]. A variety of exhibit packaging types exist and the most effective packaging for an exhibit depends on the nature of the item [2, 3]. There are likely to be variations in exhibit packaging procedures and the brand and composition of evidence bags used by crime scene examiners and between forensic science providers. While each jurisdiction and laboratory adhere to its own procedures, general packaging guidelines specify using paper evidence bags for exhibits containing biological material [3-5]. It is also recommended that breathable packaging, such as paper evidence bags, are used to package exhibits that are wet and contain biological material but cannot be fully dried at the crime scene before transport to the laboratory [4, 6].

Exhibit packaging has previously been identified to be an efficient DNA transfer vector within studies which investigated DNA transfer between exhibits and their packaging [7-10]. One previous study demonstrated that even when strict contamination minimisation procedures are applied, DNA can be transferred between the exterior of a bag containing an exhibit to the exhibit within; this is at some point during the examination process [7]. Previous studies also indicate that DNA from an exhibit can accumulate on the exterior of its packaging [8, 9]. The exact mechanisms involved in DNA transfer between the exterior of an exhibit bag and an exhibit itself is unknown, however possible mechanisms which have previously been considered include: cells being dislodged from the exterior of the bag and falling onto the exhibit; brief contact between the exhibit and the opening edges of the bag; and indirect transfer via a subsequent contact with another intermediary substrate [7-9]. One mechanism which has not been considered is the potential for DNA to

permeate the porous surface of paper evidence bags and move between the inner and exterior surface of the evidence bag. Such transfer is of concern, as it creates the potential for cross contamination between exhibits, through DNA transferred from an exhibit to its packaging [9], to the packaging of another exhibit, and onto the exhibit itself [7]. There is also limited research on the potential for DNA to be transferred between the exterior packaging of different exhibits.

The aim of this research was to determine whether DNA can permeate the porous surface of paper evidence bags and transfer between the surface of multiple evidence bags under various conditions. These aims were addressed by performing a series of highly controlled DNA transfer experiments, followed by semi-controlled experiments which simulated more realistic exhibit handling, transport and storage scenarios.

This research assists with providing a better understanding of the mechanisms which may be responsible for incidental exhibit contamination and gives insight into whether current exhibit processing procedures are still adequate given the increased sensitivity of modern DNA technologies.

4.2.3 Materials and Methods

4.2.3.1 Ethics approval

Ethics approval for this research was obtained from the Social and Behavioural Research Ethics Committee of Flinders University of South Australia and the Research and Development Committee of Forensic Science SA. Participants provided informed consent to donate the biological samples used within this study.

4.2.3.2 Sample collection

Porous substrates were sampled using DNA Tapelift Kits (Lovell Surgical Solutions, Melbourne, Australia), which were repeatedly pressed onto the substrate until the adhesiveness of the tapelift was lost. Non-porous substrates were sampled using Isohelix™ DNA/RNA Buccal Swabs (Cell Projects Ltd., Harrietsham, United Kingdom) moistened with isopropanol, which were rubbed across the designated sampling area.

4.2.3.3 Part 1- Highly controlled DNA transfer experiments

A series of highly controlled DNA transfer experiments were initially performed to assess the practical limits of the DNA to a) permeate the porous surface of a paper evidence bag and b) transfer between the exterior surfaces of bags. These experiments were designed to mimic the types of conditions that may be encountered by an exhibit when stored initially then moved through the forensic process.

4.2.3.3.1 Substrate and equipment preparation

Sections of paper evidence bags with dimensions of approximately 15 x 20 cm were cut from areas of 30 x 40 cm paper evidence bags (Saville Packaging). No areas containing folds or seams were used from the paper bags. Undyed 100 % cotton broadcloth was cut into 15 x 15 cm swatches. The pieces of paper and fabric swatches were treated with Ethylene Oxide and packaged in stacks of 50 within sealed plastic pouches.

Mori Clips, plastic suction hooks and weight blocks were soaked in a 0.5 % sodium hypochlorite solution for 2 hours, rinsed with distilled water and exposed to UV radiation for 30 minutes. Large (20 x 25 cm) and small (15 x 20 cm) pieces of glass were cleaned using a 0.5 % sodium hypochlorite solution followed by distilled water and exposed to UV radiation for 30 minutes. Where double-sided tape was used to secure substrates to the glass (experiments detailed further below), the tape was attached to the top surface of the glass prior to UV radiation.

For consistency between replicates, a template (displayed in Figure 4.1) was drawn onto each piece of glass using permanent marker, to indicate the positioning of substrates and location of saliva deposition. All equipment was cleaned between replicates using a 0.5 % sodium hypochlorite solution followed by distilled water and 30 minutes of UV exposure.

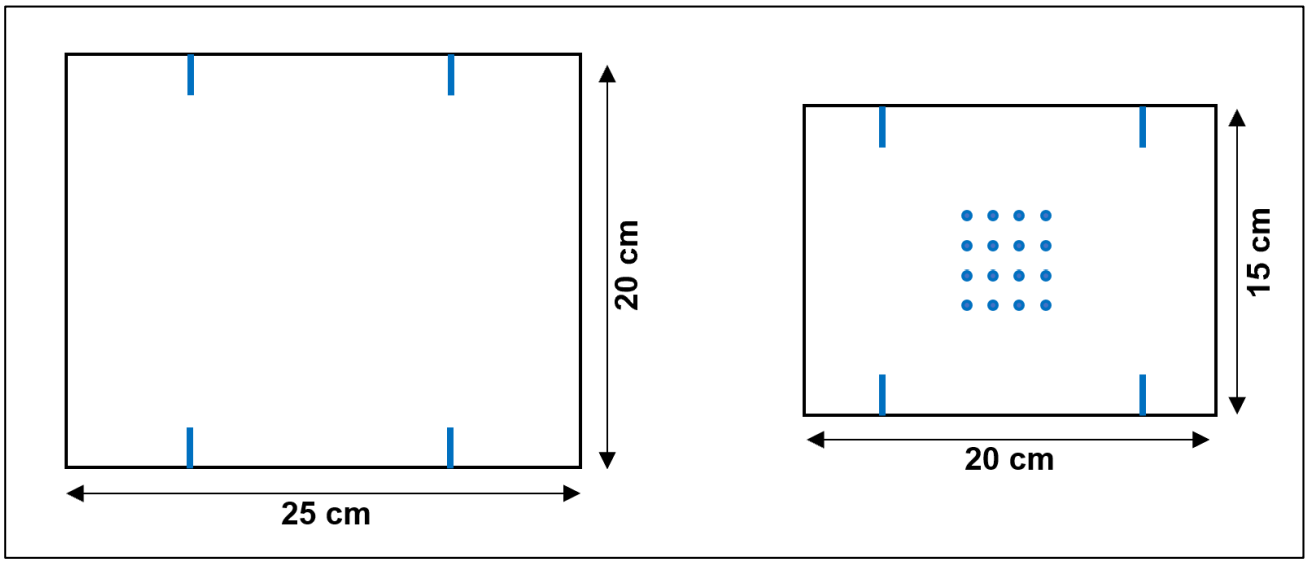

Figure 4.1. Templates drawn onto the large (left) and small (right) pieces of glass used within experiments. Marks along the edge of the large piece of glass indicate the positioning of the paper. A grid of 4 x 4 dots, which were positioned 1 cm apart, was drawn in the middle of each small piece of glass to indicate where to deposit the saliva. Marks were made along the edge to indicate the positioning of tape and the fabric.

4.2.3.3.2 Saliva solutions

Saliva was collected from donors 1-4 days before experiments were performed and stored neat in a fridge at 4°C until use. To determine an approximate measure of the amount of DNA deposited

onto substrates, aliquots of all saliva samples were extracted and quantified immediately prior to use. From each saliva sample, DNA from 20 μL was extracted in replicates of 10 by adding the solution directly into the lysis buffer during the DNA extraction process. DNA was isolated using the IQ system (Promega, Madison, WI, USA) on a Microlab® AutoLys STAR workstation (Hamilton Company, Reno, NV, USA) following the manufacturer's recommend protocol. A DNA elution volume of 60 µL was used.

DNA yields were quantified using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific, Waltham, MA, USA) on an ABI PRISM® 7500 Sequence Detection System (Applied Biosystems™) following the manufacturer's recommended protocol. The DNA concentrations of each saliva solution used within the experiments can be found in Table 4.1. Five saliva solutions were used in the experiments and the solution used within each set-up is indicated in Table 4.2.

Table 4.1. The DNA concentrations of saliva solutions used in the highly controlled DNA transfer experiments within 4.2.3.3 Part 1- Highly controlled DNA transfer experiments.

Replicate	Solution 1 concentration (ng/ µL)	Solution 2 concentration (ng/ µL)	Solution 3 concentration (ng/ µL)	Solution 4 concentration (ng/ µL)	Solution 5 concentration (ng/ µL)
1	1.576	0.879	0.065	0.0015	0.4062
2	1.536	0.820	0.061	0.0020	0.3169
3	1.843	0.832	0.067	0.0025	0.3778
4	1.571	0.706	0.077	0.0008	0.4047
5	1.824	0.999	0.055	0.0017	0.4024
6	1.730	1.151	0.061	0.0012	0.4409
7	1.564	0.803	0.081	0.0021	0.3613
8	1.578	1.076	0.130	0.0011	0.3724
9	1.844	0.968	0.049	0.0015	0.2778
10	1.491	0.944	0.078	0.0015	0.3933
Average	1.656 ± 0.044	0.918 ± 0.043	0.073 ± 0.007	0.0016 ± 0.0002	0.375 ± 0.014
Average total DNA (ng) in 500 µL	827.85 ± 21.97	458.93 ± 21.47	36.276 ± 3.60	0.798 ± 0.079	187.69 ± 6.79

Table 4.2. Summary of contact types and conditions performed in experiments. The saliva solution (as per Table 4.1) used in each experiment has been indicated.

Experiment	Contact Type	Condition	Saliva solution
4.2.3.3.3 DNA transfer through evidence bags	Friction	Wet	1
	Friction	Dry	1
	Pressure	Wet	2
	Friction	Wet	3
	Friction	Wet	4
4.2.3.3.4 DNA transfer between evidence bags	Passive	Dry	5
	Pressure	Dry	5
	Friction	Dry	5
	Movement	Dry	5

4.2.3.3.3 DNA transfer through evidence bags

A series of highly controlled experiments were performed to investigate the potential for DNA to permeate the porous surface of paper evidence bags. The potential for DNA to be transferred from an exhibit inside a bag to the exterior surface of the exhibit packaging and be detected on a substrate in direct contact with the exterior of the bag was tested. These experiments involved a substrate stack with glass plates at top and bottom, a piece of cotton dosed with saliva below the top glass plate, a section of a paper evidence bag under the cotton, and then a clean piece of cotton between the paper evidence bag and the bottom glass plate. The cotton was to mimic an item of clothing as an exhibit. The expectation is that saliva may transfer from the dosed cotton to the paper and to the clean cotton. Figure 4.2 shows the set-up used in this transfer experiment.

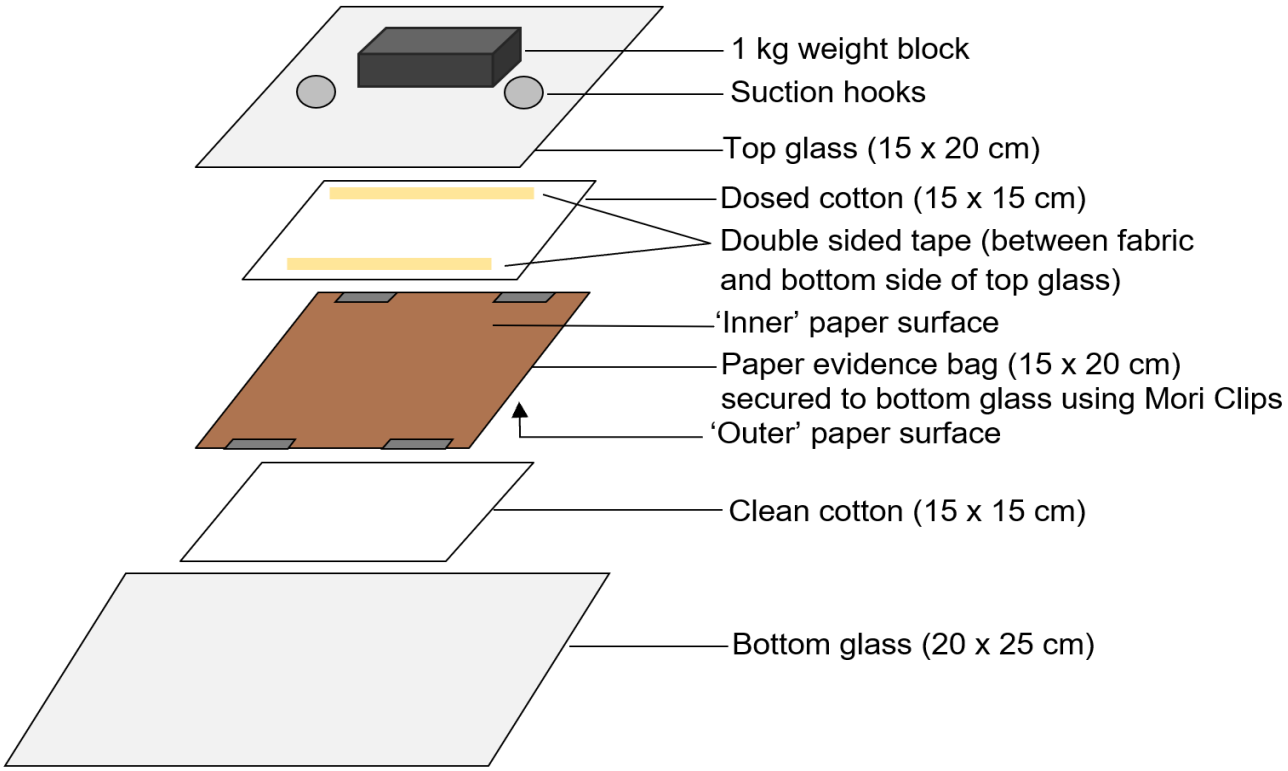

Figure 4.2. Diagram of experimental components and configuration used within the highly controlled experiments, which investigated DNA transfer through the bag. This was repeated 10 times.

A piece of double-sided tape, approximately 10 cm in length, was positioned between the two marks along each long edge of the small piece of glass. Forceps were used to remove a fabric swatch from the sterile press pouch and secure it to the small piece of glass with the double-sided tape. A fabric swatch was positioned in the centre of the large piece of glass and covered by a piece of paper from an evidence bag while ensuring that no area of the fabric was exposed. The paper was positioned between the two marks on the long edge of the glass and secured by attaching two Mori Clips along each edge. The template on the small piece of glass was visible through the fabric and used as a guide to deposit 500 μL of neat saliva, onto the attached fabric in a grid of 16 droplets as shown in Figure 4.1. Once the fabric had absorbed the saliva droplets, suction hooks were attached to the side of the glass opposite to the fabric and contact between the dosed fabric and the piece of paper was made. The suction hooks were used to assist with the movement and removal of substrates.

4.2.3.3.3.1 Contact types and conditions

Two types of contact were applied: pressure and friction. Pressure contacts involved placing a 1 kg weight onto the piece of glass at the top of the stack of substrates for 1 minute. Friction contacts involved placing a 1 kg weight onto the top piece of glass and manually moving the glass and attached fabric side to side and up and down for 1 minute. Friction contact types were tested with two different fabric conditions: dry and wet. Dry conditions involved letting the saliva on the dosed

fabric dry for 2 hours (at room temperature) before contact with the paper. Contact between the dosed fabric and paper substrates was performed 1 minute after deposition in experiments involving wet conditions. Friction contact with wet conditions was tested three times, using saliva solutions with different DNA concentrations (solutions 1,3 and 4, as per Table 4.1). The experiment where the lowest concentration of saliva was used, was performed in replicates of five. All other experiments were performed in replicates of 10. A summary of the contact types and conditions tested, and the saliva solution used within each experiment is displayed in Table 4.2.

4.2.3.3.3.2 Substrate sampling

Tapelifts were taken from three places: the surface of the paper which was in direct contact with the dosed fabric (referred to as the 'inner paper'), the surface of the paper that was in direct contact with the clean fabric (referred to as 'outer paper'), and the surface of the clean fabric swatch which was in contact with the paper. Tapelifts were submitted for DNA isolation.

4.2.3.3.4 DNA transfer between evidence bags

The potential for DNA to transfer between the surface of paper evidence bags was investigated under various conditions. These experiments involved initiating contact between a piece of paper evidence bag which was dosed with saliva and a DNA-free piece of paper evidence bag. Experiments were set up as per the diagram in Figure 4.3.

A piece of double-sided tape, approximately 10 cm in length, was positioned between the two marks along each long edge of the top piece of glass. Forceps were used to remove a piece of paper evidence bag from the sterile press pouch and secure it to the small piece of glass with the double-sided tape. A piece of paper bag was positioned in the centre of the bottom glass, between the two marks on the long edge and secured by attaching two Mori Clips along each edge. The template on the small piece of glass was used as a guide to deposit 500 μL of neat saliva, onto the attached paper in droplets. The glass with the dosed paper was placed onto the examination table and moved from side to side and up and down twice in each direction to slightly disperse each droplet of saliva. Once the saliva was dry, suction hooks were attached to the side of the glass opposite to the paper and contact between the dosed paper and the DNA-free piece of paper was initiated.

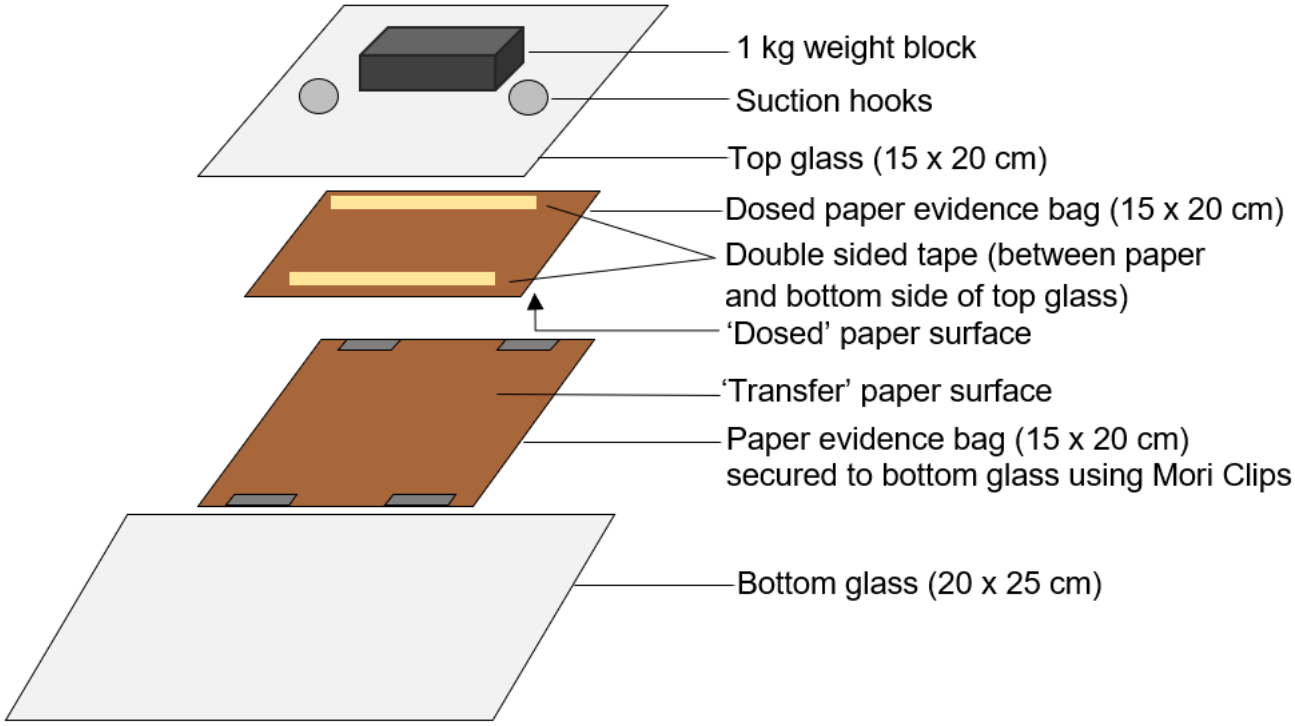

Figure 4.3. Diagram of experimental components and configuration used within the highly controlled experimental design, which investigated DNA transfer between paper evidence bags. The 1 kg weight block was only present within experiments where pressure and friction were tested.

4.2.3.3.4.1 Contact types

Four contact types were tested: passive, pressure, friction, and movement. Passive contact involved placing the dosed paper onto the DNA-free paper for 1 minute, without applying any weight or moving the substrates. Pressure contacts involved placing a 1 kg weight onto the piece of glass at the top of the stack of substrates for 1 minute. Friction contacts involved placing a 1 kg weight onto the top piece of glass and moving the glass and attached paper side to side and up and down for 1 minute. Movement contacts involved moving the glass and attached paper side to side and up and down for 1 minute without the addition of any weight. All contact types were tested in replicates of 10.

4.2.3.3.4.2 Substrate sampling

Tapelifts were taken from the surface of the paper which was dosed with saliva (referred to as the 'dosed paper') and also from the surface of the paper which was in direct contact with the dosed paper (referred to as the 'transfer paper'). With the expectation that DNA was more likely to be transferred in contacts involving friction, within setups where friction was tested, tapelifts were also taken from the other side of the dosed paper and the transfer paper. All tapelifts were submitted for DNA isolation.

4.2.3.4 Part 2- Semi-realistic DNA transfer experiments

Another series of experiments were performed to investigate the potential for DNA to permeate the porous surface of a paper evidence bag and transfer between the exterior surfaces of evidence bags under more realistic conditions. This was tested by creating mock exhibits and initiating contact between the packages of these exhibits and other substrates. These experiments were designed to replicate the types of movement and contact with other substrates that may be encountered by an exhibit as it moves throughout the forensic process. The set-up for the semi-realistic movement and friction experiments were informed by the results of the highly controlled experiments, where it was observed that transfer between the exterior surface of bags occurred under conditions that involved friction and movement.

4.2.3.4.1 DNA transfer through evidence bags

In these experiments, the potential for DNA from a mock exhibit to permeate the porous surface of a paper evidence bag and transfer onto a substrate in contact with the exterior surface of the bag was tested. Sweat stained t-shirts were used as mock exhibits and the substrate in contact with the exterior surface of the evidence bag was a plastic tub.

The inner surface of a plastic tub with the dimensions of 38.5 x 60 x 25.5 cm was cleaned with a 1% sodium hypochlorite solution, followed by distilled water and 70% (v/v) ethanol. The plastic tub was placed on top of a flat top trolley typical of that used to transfer evidence bags between storage and evidence recovery laboratories. A swab was also collected from the centre portion of the inside of the plastic tub.

A 100% cotton t-shirt, that was previously worn by an individual while running for approximately 30 minutes, was placed into a paper evidence bag (dimensions 30 x 40 cm) (Saville Packaging). Prior to packaging, the shirt was stored within an unsealed plastic bag for approximately 4 hours. The evidence bag was sealed using packaging tape and a tapelift was taken from a 17 x 30 cm area of the bag indicated in Figure 4.4.

Figure 4.4. Front and back of mock exhibit package. The area used to sample the bag is shaded in red.

The sampling area selected on the sealed evidence bag was free from folds and seams, which may impact the ability for the surface of the bag to make uniform contact with the tub. To minimise the risk of collecting DNA which was present on the packaging tape used to seal the bag, the side of the bag which contained the evidence seal was not sampled.

The mock exhibit was placed inside the plastic tub with the previously sampled area of the bag in contact with the area of the tub that had been previously swabbed. The trolley was pushed by an individual while walking for 5 minutes, to simulate the movement involved in transporting exhibits. The mock exhibit was then removed from the trolley and placed onto examination paper and the previously sampled portions of the evidence bag and tub were re-sampled. The mock exhibit was placed back into the tub in the same orientation as it was previously, and the tub was covered and left without movement for 2 hours. After 2 hours, the bag containing the mock exhibit was removed from the tub and a further sample was taken from the previously sampled sections of the bag and the plastic tub. Gloves were changed between handling of the exhibit, exhibit packaging, plastic tub, trolley and prior to taking samples. This experiment was repeated three times using a new cotton t-shirt for each setup. The plastic tub was thoroughly cleaned with a 1% sodium hypochlorite solution, followed by distilled water and 70% (v/v) ethanol between each experiment.

4.2.3.4.2 DNA transfer between evidence bags

Within these experiments, the potential for DNA to transfer between the exterior surface of the package of mock exhibits was investigated. This was tested by placing two packaged mock exhibits into a plastic tub on a flat top trolley with the exterior surface of the packages contacting each other. Prior to contact, the exterior surface of the packaging of one mock exhibit was handled by an individual without the use of PPE. Samples were taken from the exterior packaging of both exhibits and the plastic tub before and after contact was initiated. The inner surface of a plastic tub with dimensions of 38.5 x 60 x 25.5 cm was cleaned with a 1% sodium hypochlorite solution, followed by distilled water and 70% (v/v) ethanol. The plastic tub was placed on top of a trolley and the centre section of the inside of the plastic tub was swabbed.

Two previously washed cotton t-shirts were used as mock exhibits within these experiments. The t-shirts were packaged separately inside of paper evidence bags (dimensions of 30 x 40 cm) (Saville Packaging). Evidence bags were sealed using packaging tape and a tapelift was taken from the bottom area of each bag highlighted within Figure 4.4. The area sampled measured approximately 17 x 30 cm; this was on the side of the bag that did not contain the evidence seal. One packaged t-shirt (exhibit A) was placed into the plastic tub with the area of the bag which was previously sampled facing upwards. The package of the second t-shirt (exhibit B) was handled by an individual for 2 minutes before being placed inside of the plastic tub, with the section of exhibit B that had previously been sampled in contact with exhibit A. The handler was not wearing PPE and had not washed their hands within 30 minutes prior to contact with the exhibit packaging. Contact included picking the exhibit up by the sides of the bag, rotating the exhibit, as if to inspect the whole exterior surface of the package and holding onto the package for the remainder of the allocated time.
The trolley containing the exhibits was pushed by a different individual while walking at normal pace for 5 minutes, to simulate the process involved in transporting exhibits. Exhibit B was then removed from the trolley and a sample was taken from the same portion of the bag and tub that had previously been sampled. The bag was placed back into the tub in the same orientation as it was previously, the tub was covered and left without movement for 2 hours. After 2 hours another tapelift was taken from the previously sampled area of the evidence bag and the plastic tub. All tapelifts were submitted for DNA analyses.

4.2.3.5 DNA Analysis

All stages of DNA analyses were performed using in-house validated protocols at FSSA. DNA samples were extracted using the DNA IQ system (Promega, Madison, WI, USA) on a Microlab® AutoLys STAR workstation (Hamilton Company, Reno, NV, USA), with an elution volume of 60 µL. DNA yields were quantified using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific, Waltham, MA, USA) on an ABI PRISM® 7500 Sequence Detection System (Applied

Biosystems™). DNA was amplified using the GlobalFiler™ PCR kit on a ProFlex™ Dual 96-Well PCR System (Thermo Fisher Scientific). Cycling conditions were as per manufacturer's recommendations for 29 cycles, with 400 pg of DNA or a maximum DNA volume of 15 µL if 400 pg was not available, in a final volume of 25 µL. Amplified DNA fragments were resolved using an ABI 3500xl Genetic Analyser (Applied Biosystems™).

4.2.3.6 DNA Profile Interpretation and Analysis

Electropherograms were analysed using GeneMapper® ID-X Software V1.6 (Thermo Fisher Scientific). For the highly controlled transfer experiments in Part 1 (4.2.4.1), an allele analytical threshold (AT) of 50 relative fluorescence units (RFU) was used to analyse electropherograms. To maximise the amount of usable information, electropherograms with visible peaks below the AT of 50 RFU were reanalysed with a reduced AT of 20 RFU. Within 4.2.4.1.1 (DNA transfer through bags), 36 electropherograms were reanalysed and within 4.2.4.1.2 (DNA transfer between bags), 12 electropherograms were reanalysed using a reduced AT. For the semi realistic transfer experiments within Part 2, all electropherograms were analysed using an AT of 20 RFU.
The minimum number of individuals required to reasonably explain each DNA profile was determined using maximum allele count and taking into account peak heights. STRmix™ V2.9.1 was used to deconvolute DNA profiles into individual contributor proportion, using in-house derived settings. Profiles generated from all electropherograms that contained at least one allele were compared to reference profiles from DNA donors. For each comparison, a likelihood ratio (LR) was calculated using the opposing H1 and H2 propositions:

H1) the staff member and (N-1) unrelated individuals are the sources of DNA

H2) N unknown individuals, unrelated to the staff member are the sources of DNA

(where N is the number of profile contributors).

The LR value considered was the sub-source LR value, calculated using an Australian Caucasian allele frequency database [11].

4.2.3.7 Data Analysis

Samples that yielded an undetectable DNA quantity were given a value of 0.00005 ng (-4.3 on a log scale), which is half of the limit of detection of the Quantifiler™ Trio DNA Quantification Kit. For statistical comparisons, DNA amounts were log transformed. All statistical analyses were performed using R Software V4.1.2 and a significance level of $p < 0.05$.

For experiments within 4.2.3.3.3, a linear regression model was used to assess the relationship between the amount of DNA detected (log10 ng of DNA) and the different samples, contact types and conditions with consideration of the estimated amount of DNA that was initially deposited. The

average concentration (ng/µL) of DNA within the quantified aliquots of each saliva solution was calculated and then multiplied by 500 (µL of solution deposited onto the dosed fabric), to estimate the amount of DNA (in ng) that was initially deposited onto dosed substrates within each experiment. Post-hoc comparisons using estimated marginal means were performed. Pairwise comparisons were made using the Tukey adjustment method to control for multiple comparisons. Estimated marginal means were calculated for combinations of sample type for each contact and condition combination and adjusted for the effect of the average amount of DNA deposited. The pairwise differences in the amount of DNA detected (log10 ng of DNA) between the sample types (paper inner, paper outer and fabric) within each combination of contact type and condition (friction/dry, friction/wet, pressure/wet) were assessed.

For the experiments within 4.2.3.3.4, where a saliva solution with the same concentration was used across all experiments, the DNA detected on each substrate for each contact type was compared using a Wilcoxon signed-rank test. This test was used due to the paired nature of samples taken from each of the substrates. A linear model was used to test whether the difference in DNA on substrates varied across contact types. Post-hoc analysis was performed using estimated marginal means to compare the difference in DNA amounts between the substrates across each contact type.

Due to the limited number of each type of sample and the variation in each sample observed across replicates within the semi-realistic transfer experiments in 4.2.3.4, statistical analysis was not performed.

4.2.4 Results

4.2.4.1 Part 1- Highly controlled DNA transfer experiments

4.2.4.1.1 DNA transfer through bags

Samples taken from the inner and outer surfaces of a piece of paper bag positioned between a fabric swatch dosed with saliva and a DNA-free fabric swatch were quantified. For the three combinations of experimental conditions tested, the quantities of DNA (ng) present on the inner surface of a piece of paper bag, which was in direct contact with the dosed swatch, the outer surface of the paper bag and also the clean piece of fabric, are displayed in Figure 4.5.

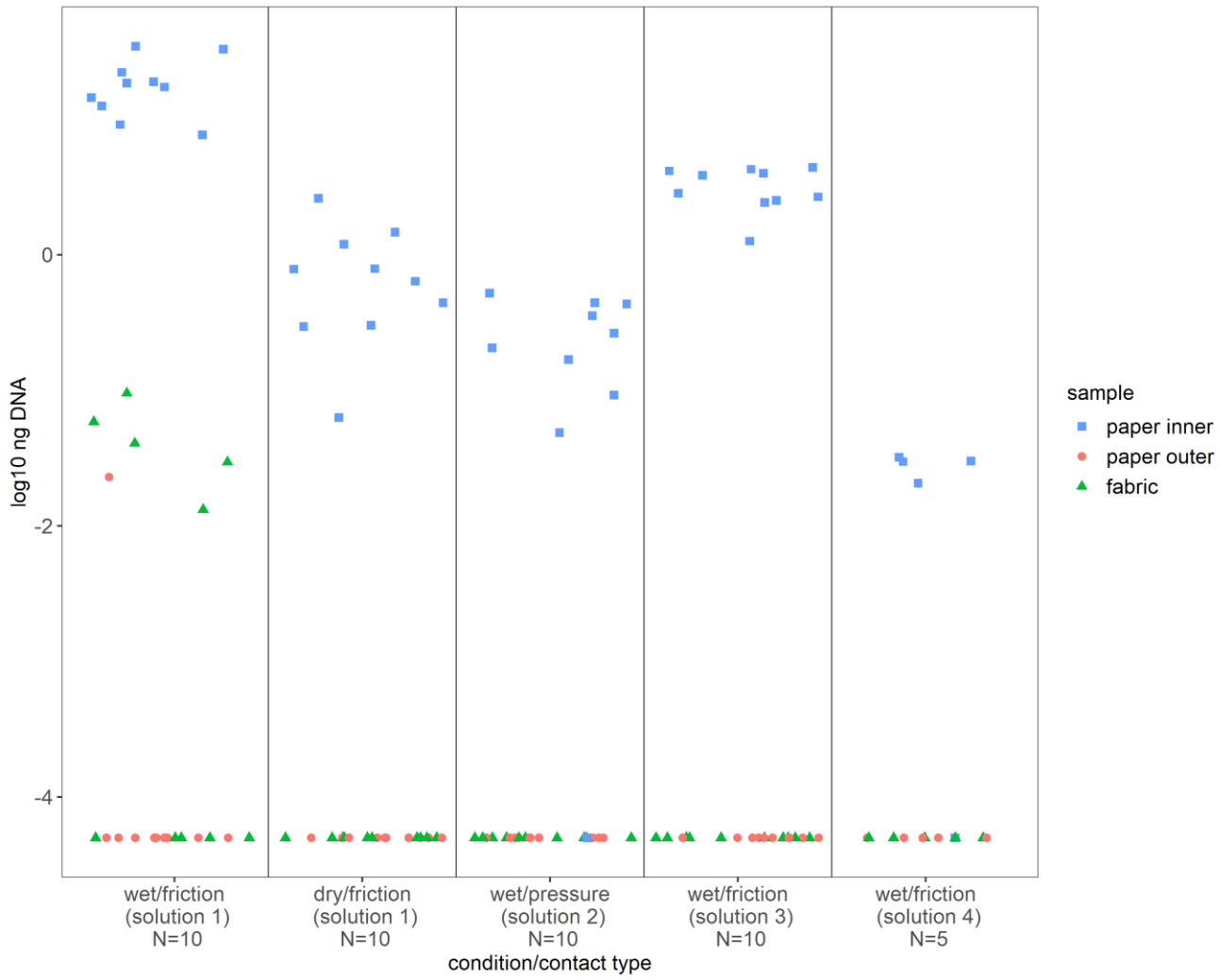

Figure 4.5. The log 10 DNA quantities (ng) recovered from samples taken from the inner paper surface, outer paper surface and fabric swatch for each condition and contact type tested. The three combinations of conditions tested were: wet saliva dosed substrate with friction applied; dry saliva dosed substrate with friction applied and wet saliva dosed substrate with pressure applied. The experiment that involved wet conditions with friction was tested three times, with less concentrated saliva solutions (solutions 3 and 4) used within the second and third experimental setups, compared to the solution used within the first setup (solution 1).

Of the 135 samples taken, 86 were reported as having an undetected DNA quantity. There were two samples taken from the inner paper surface that yielded undetected DNA samples. One of these was within an experiment where pressure was applied to a wet substrate and the other was where a wet substrate was tested with friction and the lowest concentration saliva solution (solution 4) was used. Undetected DNA quantities were yielded from all samples taken from the outer paper surface and fabric in experiments where pressure was applied and the substrate was wet, and where friction was applied, and the substrate was dry. Samples taken from the outer paper surface and fabric within the experiments where the substrate was wet, friction was tested and the DNA solutions with the lowest concentrations (solutions 3 and 4) were used also yielded undetected DNA quantities.

Highest DNA quantities were reported from samples taken from the inner paper surface within experiments where friction was applied, the substrate was wet and saliva solution 1 (highest DNA concentration) was used. These values ranged from 7.70 - 34.57 ng. In comparison, when the same solution was used, but friction was combined with dry conditions, DNA quantities yielded from the inner paper surface samples ranged from 0.06 – 2.61 ng. The DNA quantities from the inner paper samples within the experiments with friction tested and a wet substrate, but less concentrated saliva solutions (solutions 3 and 4) used, ranged from 1.26 - 4.41 ng for solution 3 and undetected (<0.001 ng/µL) to 0.03 ng for solution 4. In experiments where pressure was applied, and a wet substrate was used, these samples yielded DNA quantities ranging from undetected (<0.001 ng/µL) to 0.52 ng.

Linear regression analysis indicated that the amount of DNA detected was significantly influenced by sample type (specifically inner paper samples), contacts which involved a wet substrate and friction, and the amount of DNA initially deposited. Overall, the model was statistically significant ($p < 2.20\times10^{-16}$) and explained approximately 87% of the variance in the quantities of DNA recovered (Adjusted R^2 value= 0.87).

The amount of DNA initially deposited was determined to be a significant predictor of the amount of DNA detected within samples ($p= 4.53\times10^{-11}$). For all combinations of contact type and condition tested, the sample taken from the inner paper surface consistently yielded significantly higher DNA amounts when compared to the samples taken from the outer paper surface and the fabric ($p <0.0001$).

Although not significant, for wet conditions where friction was applied, the difference in the DNA amounts yielded from the outer paper surface and the fabric approached significance ($p = 0.072$), which suggested there is a possible increase in the amount of DNA detected in samples taken from the fabric compared to the outer paper. Within all other setups, the difference between the DNA quantities in samples taken from the outer paper surface and the fabric was not significant (all $p= 1.0$).

When friction was applied and the substrate was wet, the amount of DNA detected was significantly higher than when friction was applied and the substrate was dry ($p= 0.0122$). No significant interaction effects were observed between the sample type and the contact type and condition (all p-values > 0.1), indicating that the influence of the nature of contact on DNA amount was consistent across the difference sample types for all setups.

At least one allele was observed within the electropherogram generated from 74 of the 135 samples. The majority of profiles were assigned a NoC of 1 (N=70), with the remaining four assigned a NoC of 2. The 74 DNA profiles generated from samples taken within all experimental set ups were compared to the DNA profile from the saliva donor. In 81% of comparisons, an

inclusionary LR (LR>1) was generated to the saliva donor. The LR yielded for an unambiguous single source profile from the saliva donor within these experiments was 1.17×10^{27}. The log10 LRs generated in comparisons between the DNA profile from the donor of the saliva and DNA profiles generated from samples within all experiments are displayed within Figure 4.6.

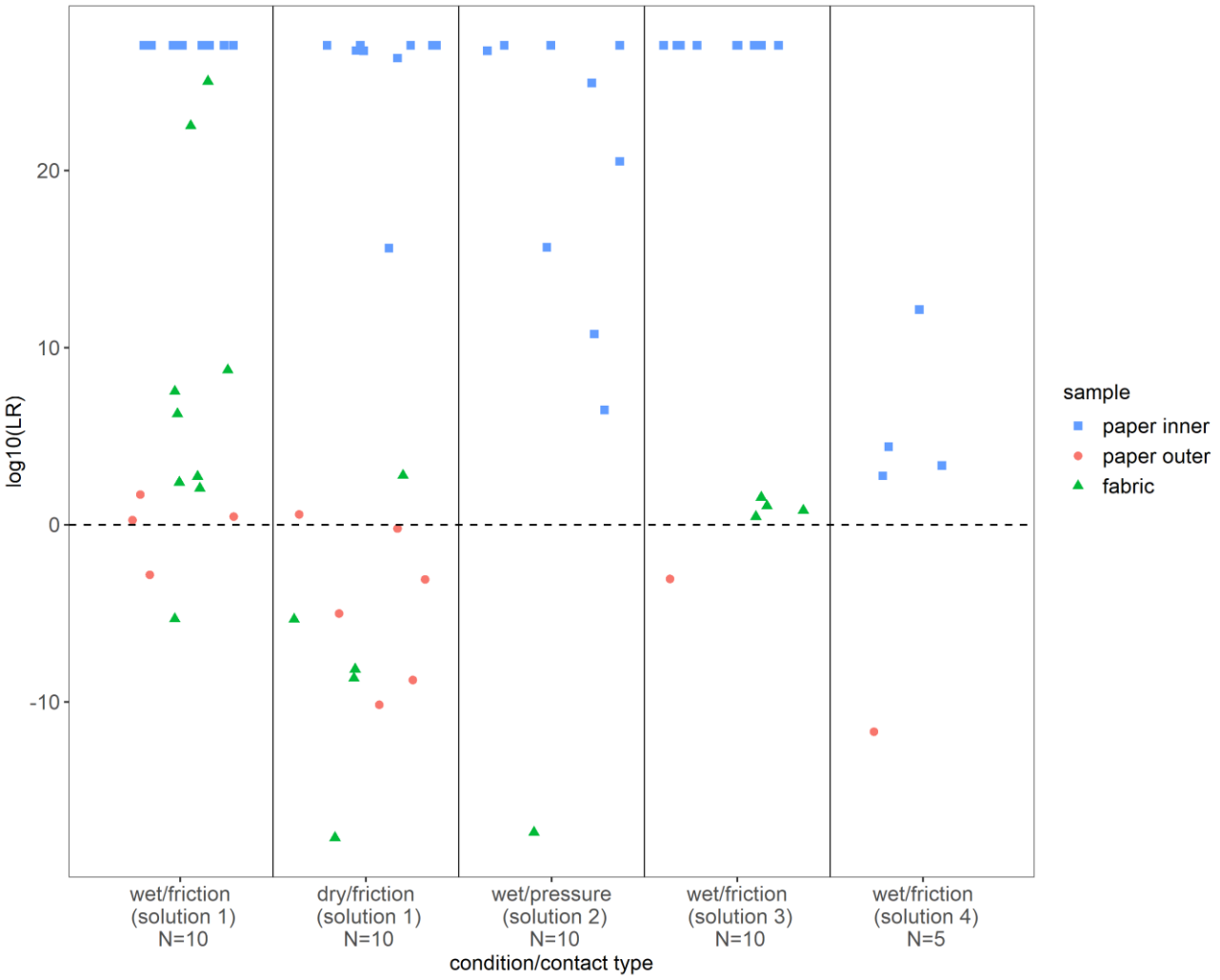

Figure 4.6. log10(LR) generated in comparisons to the saliva donor in samples taken from the inner paper surface, outer paper surface and fabric swatch for each condition and contact type tested.

Except for the two samples that yielded an undetected DNA quantity, all samples taken from the inner paper surface yielded an inclusionary LR to the saliva donor. Despite having undetected DNA quantities, DNA profiles were generated from 25 samples taken from the fabric and outer surface of the paper. Eleven of these profiles generated a low inclusionary LR to the saliva donor. The remaining 14 profiles generated an LR which favoured the exclusion of the saliva donor as the source of the DNA.

4.2.4.1.2 DNA transfer between bags

The DNA quantities yielded from samples taken from the paper dosed with saliva, and the section of paper in direct contact with the dosed paper for each of the contact types, are displayed in Figure 4.7.

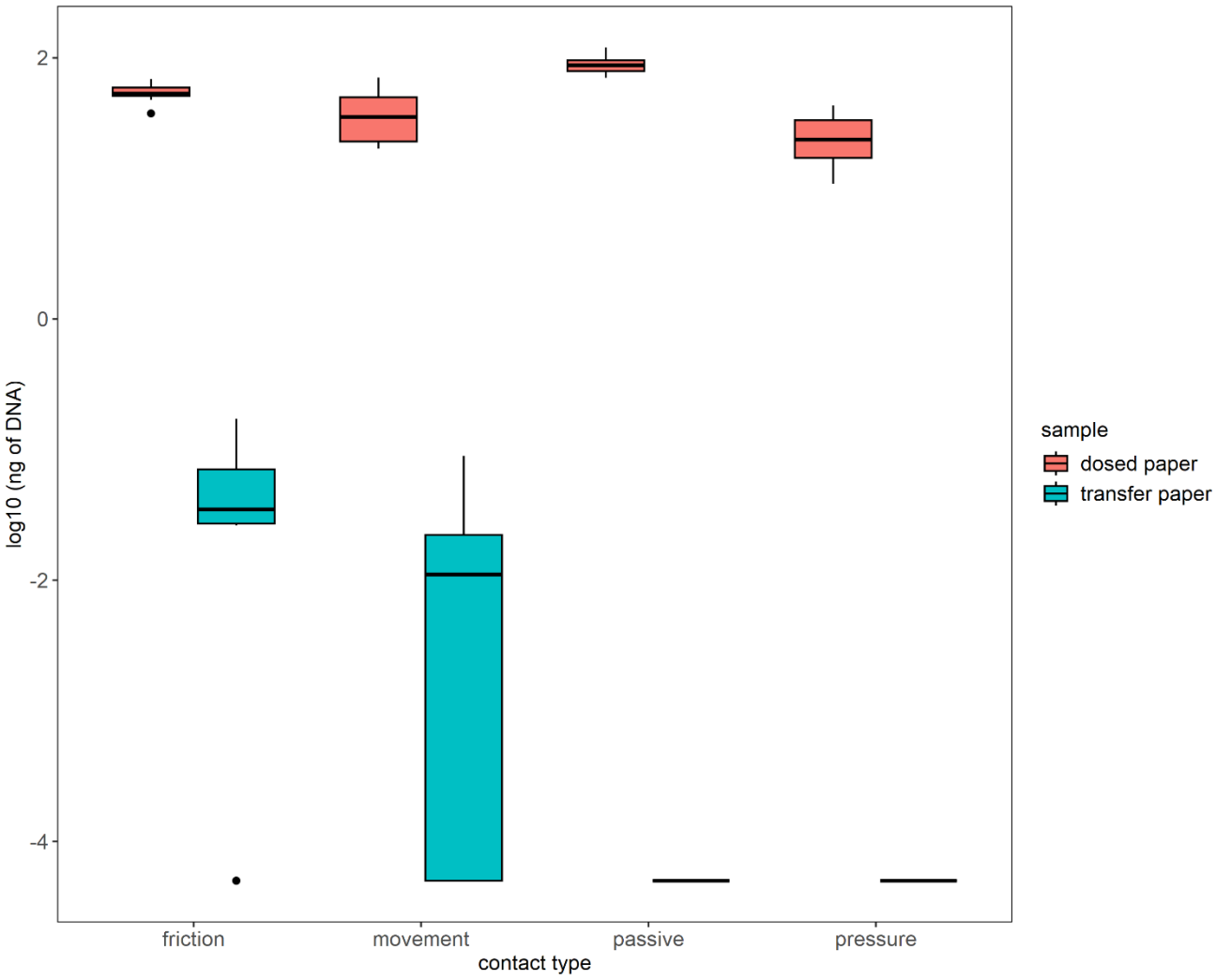

Figure 4.7. DNA quantity detected on the dosed paper and the 'transfer paper' which was in direct contact with the dosed substrate for different contact types (N= 10). The dots indicate outliers. Undetected DNA quantities were assigned a value of 0.00005 ng (-4.3 on a log 10 scale).

There is a distinctive difference between the DNA amounts obtained from the dosed paper, onto which DNA was added, compared to the section of paper in direct contact with the dosed paper. The Wilcoxon signed-rank test indicated these differences in DNA amounts between substrates were significant for all contact types (all p= 0.00195). Transfer from paper to paper appeared to be minimal for setups where contact was made without any sort of movement between the substrates (see figure 4.3 for experimental setup). All samples taken from the other side of each piece of paper within setups where friction was tested yielded undetectable DNA quantities.

The linear model showed significant effects on DNA amount for both substrate and contact type, as well as significant interaction effects between substrate and contact type. The model was

statistically significant ($p < 2.2 \times 10^{-16}$) and explained approximately 91% of the variation in DNA amount. Substrate type had a pronounced effect on the amount of DNA, with dosed paper samples yielding significantly more DNA than the paper in direct contact ($p < 2.0 \times 10^{-16}$). Contact type also influenced DNA, with significantly higher amounts of DNA yielded from the dosed paper in passive contact, compared to friction ($p= 1.8 \times 10^{-11}$), movement ($p = 0.0009$) and pressure ($p = 5.25 \times 10^{-9}$).

Significant interaction in the form of friction indicated that the impact of contact type on amount of DNA detected varied by substrate in the different experiments. For example, the effect of passive contact on amount of DNA yielded in samples taken from the dosed paper was significantly reduced in samples taken from the transfer paper ($p = 3.15 \times 10^{-7}$), and similar patterns were seen for movement ($p = 0.0168$) and pressure ($p = 1.31 \times 10^{-5}$) contacts.

Post hoc analysis indicated that the difference in DNA between dosed and transfer paper varied significantly for all contact types (for all $p< 0.0001$). The difference in the DNA amounts between the substrates in friction setups was less pronounced compared to other contact types and more pronounced for passive contacts. DNA recovery from dosed paper varied depending on contact type, whereas transferred substrates yielded negligible DNA amounts in all contact types.

When compared to the DNA now isolated from the previously DNA-free paper, it is clear the greatest factor affecting DNA transfer is friction. This contrasts with passive and pressure where no DNA was detected. Of the 10 replicates, where movement was tested, there was a spread of DNA that transferred from minimal to comparable with friction.

At least one allele was observed within the electropherogram generated from 57 of the 90 samples. An inclusionary LR (LR>1) was generated to the saliva donor in 54 of the 57 comparisons. The resulting LRs from the DNA isolated from both the dosed paper and the piece of evidence bag paper in direct contact are shown in Figure 4.8.

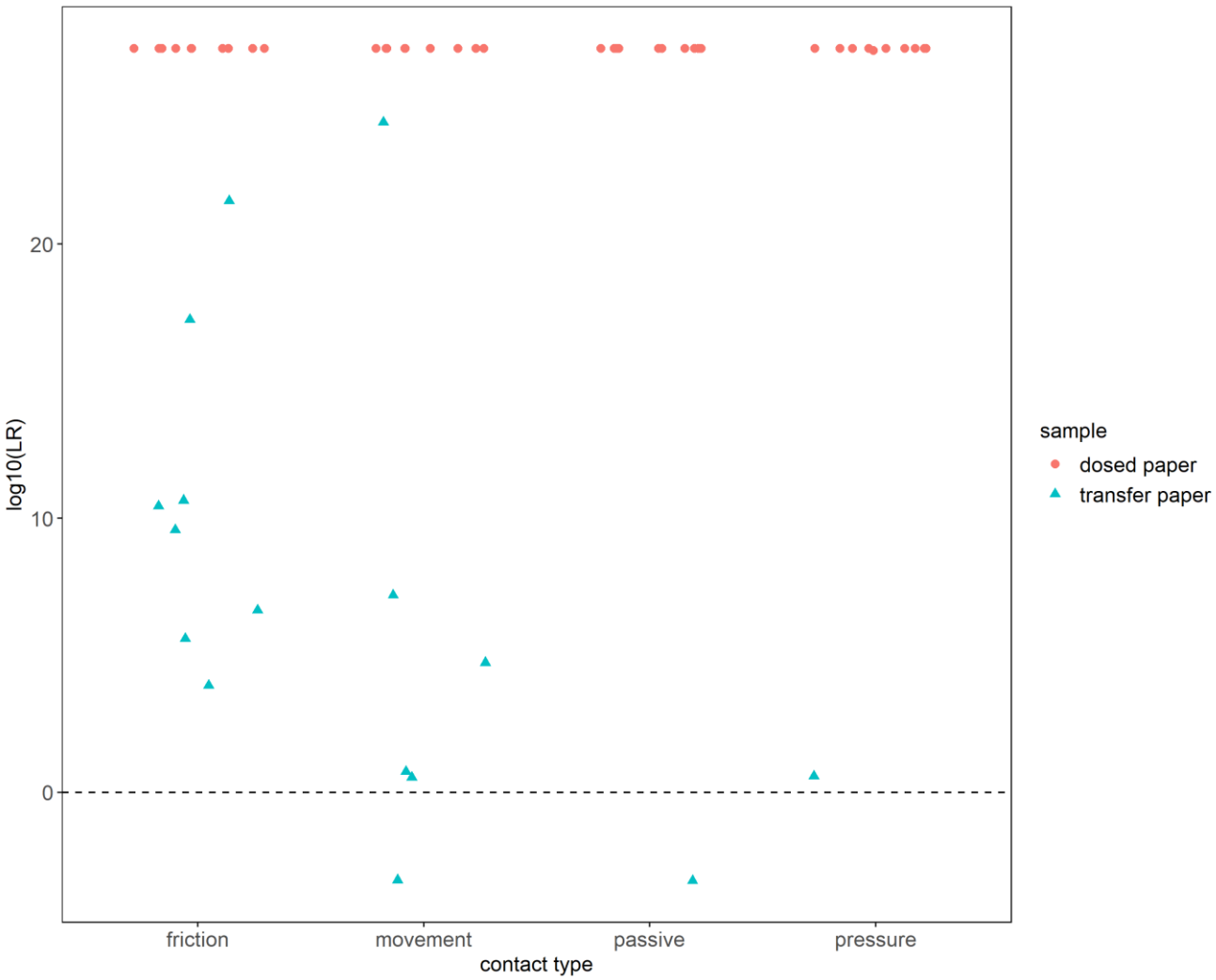

Figure 4.8. Log10 LR generated between each sample (n=10) and the DNA donor for each contact type tested.

The LR generated for an unambiguous single source profile from the saliva donor within these experiments was 1.40×10^{27} A single comparison within the sample taken from the other side of the transfer paper yielded a LR of 0.00059, favouring exclusion of the DNA donor. The samples taken from both sides of the transfer paper for this replicate yielded an undetectable DNA quantity. Low LR values were obtained from the DNA transferred to the previously DNA-free paper for both the passive and pressure experimental setup. The LR from the eight friction samples, where a DNA profile was generated, show a spread from a low LR for two samples to a high LR for two samples, with the other six samples generating LRs above 1000. These data support the premise that little or undetectable DNA is transferred between paper when the biological material was dried and applying only a weight without movement (or no weight). Friction and movement however can result in DNA transfer between paper.

4.2.4.2 Part 2- Semi-realistic DNA transfer experiments

The results of the highly controlled experiments led to the experimental set-ups to test the potential for DNA to permeate through and transfer between the surface of paper evidence bags under semi realistic conditions. This was tested using mock exhibits packaged within paper evidence bags and initiating various types of contact between substrates.

4.2.4.2.1 DNA transfer through bags

Samples were taken from the outside of the package of a mock exhibit before and after the item was placed into the plastic tub and movement was initiated, and again after storage in the tub for 2 hrs. Samples were also taken from the tub at each of these time points. The paper evidence bags and plastic tub used within these experiments were likely to contain some level of DNA prior to use, therefore samples were taken before contact between substrates to establish a baseline DNA level.

The DNA within samples taken from the exhibit package and the plastic tub were quantified and these values are displayed in Table 4.3. The NoC was assessed within the electropherograms generated from all samples and LRs were calculated in comparisons between the DNA profiles generated from samples and the DNA profile from the t-shirt donor. The apparent NoC and resultant LRs are provided within Table 4.3.

Table 4.3. DNA quantities and NoC within samples taken from exhibit packaging and the plastic tub in experiments where the potential for exhibit DNA to permeate the bags' surface and transfer onto another substrate was tested. Undetected DNA quantities have been represented with a value of 0. The LR yielded between the comparison of DNA profiles generated from samples and the DNA profile from the t-shirt donor are displayed, with LRs >1 bolded.

Replicate	Sample	DNA (ng)	NoC	LR to t-shirt donor
1	bag_before	0	0	NA
	tub_before	1.0188	2	1.78×10^{-2}
	bag_ movement	0	0	NA
	tub_movement	0.0203	1	7.92×10^{-21}
	bag_time	0	0	NA
	tub_time	0.0454	2	**1.31**
2	bag_before	0	1	**5.79**
	tub_before	0.0088	1	5.47×10^{-4}
	bag_movement	0	1	1.33×10^{-6}
	tub_movement	0.0113	1	0
	bag_time	0	1	3.72×10^{-4}
	tub_time	0.0175	1	**3.54**
3	bag_before	0	0	NA
	tub_before	0	1	NA
	bag_movement	0	1	**1.25**
	tub_movement	0	1	5.97×10^{-4}
	bag_time	0	0	NA
	tub_time	0	0	NA

In replicates 1 and 2, all samples taken from the tub yielded low DNA amounts, while all samples taken from the exterior surface of the exhibit bags within these experiments resulted in undetected DNA quantities. Very low inclusionary LRs were generated to the t-shirt donor in four samples. Two of these LRs were generated in samples taken from the plastic tub (after the exhibit was stored within the tub for two hours). The remaining two inclusionary LRs were generated within samples taken from the bag before the item was placed into the tub (within replicate 2) and within a sample taken after movement was initiated (replicate 3). The sample taken from the tub after movement was initiated within replicate 2 yielded an exclusion to the t-shirt donor. All other samples within these experiments generated LRs that favoured the exclusion of the t-shirt donor as a sample donor.

4.2.4.2.2 DNA transfer between bags

Samples were taken from the exterior packages of two mock exhibits and the plastic tub at two different time points within each experiment; before and after contact with initiated between the substrates. The DNA within samples taken from the exhibit packages and the plastic tub were quantified and are displayed in Table 4.4. The NoC within electropherograms generated from all

samples, the resultant LR for comparison between the DNA profile generated from samples, and the DNA profile from the individual who handled the bag are displayed in Table 4.4.

Table 4.4. DNA quantities recovered from samples taken from evidence bags and the plastic tub before and after contact. These experiments tested the potential for DNA to transfer between the surface of exhibit bags and other substrates. Undetected DNA quantities have been represented with a value of 0. The apparent NoC within the electropherogram and the LR yielded between the comparison of DNA profiles generated from samples and the DNA profile from the t-shirt donor are displayed, with LRs >1 bolded.

Replicate	Sample	DNA (ng)	NoC	LR to bag handler
1	tub_before	0	0	NA
	tub_after	0	1	6.32×10^{-3}
	held bag_before	0	0	NA
	held bag_after	0.0581	2	**1.71×10^{17}**
	bag_before	0	1	**1.82**
	bag_after	0	1	**6.85**
2	tub_before	0	0	NA
	tub_after	0.0530	1	2.15×10^{-32}
	held bag_before	0	0	NA
	held bag_after	0	1	1.59×10^{-5}
	bag_before	0	0	NA
	bag_after	0	0	NA
3	tub_before	0	0	NA
	tub_after	0.0301	1	0
	held bag_before	0	1	**1.57**
	held bag_after	0.0736	2	**1.03×10^{21}**
	bag_before	0	0	NA
	bag_after	0	2	7.62×10^{-3}

Within replicates 1 and 3, samples taken from the bag that had been directly handled by the DNA donor yielded detectable levels of DNA and generated high LRs when compared to the DNA profile from the bag handler. The same sample taken within replicate 2 yielded an undetectable DNA quantity and the LR generated from the resultant DNA profile favoured exclusion of the bag handler.

In replicates 2 and 3, detectable levels of DNA were also yielded from sample taken from the plastic tub after contact with the exhibit bags. Comparison between the resultant DNA profiles generated from these samples and the DNA profile from the bag handler, resulted in LRs which excluded or favoured exclusion of the bag handler. All other samples taken within these experiments yielded undetectable DNA quantities.

Despite yielding undetected DNA quantities, in replicate 1, low inclusionary LRs were generated to the bag handler in both samples taken from the bag that was placed into the tub without being handled by the experimental DNA donor. A low inclusionary LR was also generated between the sample taken from the handled bag prior to contact and the bag handler. For all other samples where the resultant DNA profile was compared to the bag handler, the LRs yielded favoured the exclusion of the bag handler as a DNA donor.

4.2.5 Discussion

Investigations into DNA transfer between exhibits and their packaging provide valuable insight into best practice for the packaging, storage and processing of forensic exhibits. In addition to expanding the current body of knowledge on DNA transfer, persistence, prevalence and recovery (TPPR), such research is vital to assess and improve current exhibit handling and storage procedures. This study aimed to determine whether DNA can permeate the porous surface of paper evidence bags, transfer between the surfaces of multiple evidence bags, and onto other substrates under different conditions. To address these aims, a two-part experimental approach was adopted. A series of highly controlled DNA transfer experiments were performed first, followed by semi-realistic experiments which were designed to better simulate realistic handling, transport and storage scenarios.

4.2.5.1 Part 1- Highly controlled DNA transfer experiments

Throughout the controlled experiments in Part 1 of this study, the potential for DNA to be transferred to the surface of a paper evidence bag during various modes of direct contact with pieces of cotton and other paper evidence bags was demonstrated. Within these experiments, several factors were shown to influence the amount of DNA detected on a previously DNA-free substrate after contact with a substrate dosed with saliva. Several trends observed within the controlled experiments were consistent with the findings of previous research.
In previous studies, the secondary transfer of DNA from a surface dosed with saliva has been demonstrated using an assortment of contact scenarios and substrate types [12-15]. While paper substrates were not tested within these previous studies, it was expected that paper would behave in a similar manner to the other porous substrates that were tested, and therefore similar overall trends would be observed. There are no existing studies where DNA transfer between or through paper surfaces has been tested.

In the experiments where the potential for DNA to transfer through the surface of an evidence bag was tested, the amount of DNA detected in samples depended on the amount of DNA initially deposited, the type of contact and whether the saliva was wet or dry at the time of contact. For all combinations of contact type and condition tested, samples taken from the inner paper surface consistently yielded significantly higher DNA amounts than the outer paper surface and fabric. Due to direct contact with the cotton dosed with saliva, it was expected that these samples would yield

the highest DNA quantities and generate an LR to the saliva donor. The observation that DNA can be transferred to a paper evidence bag in direct contact with a piece of cotton dosed with saliva supports findings within other studies where DNA transfer between an exhibit and a paper evidence bag surface has been observed [7-10]. DNA transfer was observed between a range of items packaged within paper evidence bags and the inside surface of the evidence bag by Goray et al. [10] and Stella et al. [16]. In Mercer et al. [8] contact between a sweaty t-shirt and the exterior of the exhibit bag surface resulted in DNA transfer to the exterior surface of the packaging. There are several studies where DNA transfer between an exhibit and the exterior of an exhibit packaging explains the findings in the study, but the exact transfer mechanism was unknown [7-9, 17].

The comparison of the DNA profile from the saliva donor and all samples taken from the inner paper surface that yielded detectable DNA quantities yielded an LR that favoured inclusion of the saliva donor. According to the verbal equivalent scale within Evett and Weir [18], LRs of this magnitude provides extremely strong support for the inclusion of the individual as a sample donor. Except for the experiments where the lowest concentration saliva solution was used, a full profile from the saliva donor was yielded from most inner paper samples in experiments where friction was applied. In the experiments where pressure was applied, the magnitude of LRs yielded from the inner paper samples varied, but all samples except one still provided extremely strong support for the inclusion of the saliva donor as a contributor to the profile.

Two samples taken from the inner surface of the paper bag that were in direct contact with the cotton dosed with wet saliva unexpectedly yielded undetected DNA quantities. The source of such variability in the results is unknown, but possible explanations include variation in the amount of DNA in the deposited saliva or inefficiency in the DNA transfer, collection or extraction. Due to the inconsistent viscous nature of saliva [19, 20], it is possible that higher amounts of DNA rich cells, such as epithelial cells and leukocytes [21-23], are clustered within the more viscous portions of the solution, instead of being evenly suspended throughout the entire solution. This may have resulted in less DNA being deposited onto some swatches and consequently less DNA being available for transfer. Due to the absorbent nature of porous substrates [13, 15], it is also likely that some portion of DNA transferred was retained by the paper and not collected by the tapelift [24]. It is also possible that the DNA was collected and retained by the tapelift or lost during the DNA extraction process [25]. Verdon et al. [15] have previously demonstrated that when the same collection and extraction methodology is utilised and the same substrate is sampled, the amount of DNA retrieved can differ significantly.

As the DNA concentration of the saliva deposited onto the cotton decreased, lower amounts of DNA were detected on the inner paper surface. This was reflected in the comparatively lower LRs that were generated between the saliva donor and these samples. A lower DNA concentration means less DNA is deposited, resulting in less DNA available for transfer, therefore this result was

expected. For experiments where the same saliva solution was used and friction was tested, a direct comparison could be made between the DNA quantities yielded in dry and wet transfer conditions. Lower quantities of DNA were detected in samples taken from the inner paper surface when the substrate was dry, compared to when the substrate was wet. This result is consistent with previous studies which indicate that more DNA is transferred between substrates in contacts where the source of DNA is wet at the time of contact [12-14]. Higher amounts of DNA were detected in contacts where friction was applied, compared to pressure and passive contacts. These findings are also consistent with previous studies [12, 13].

It has previously been demonstrated that DNA from various biological fluids can be detected on the opposite side of different porous fabrics to where it was originally deposited [26]. Given the porous nature of paper evidence bags, it was expected that some portion of the wet saliva may permeate the surface of the bag. If transfer through the surface of the paper evidence bag was observed, it was expected that DNA would be detected in samples taken from the outer surface of the evidence bag and the DNA free fabric in direct contact with the outer bag surface. DNA was detected on the outer surface of the paper bag and fabric within five experiments where the highest concentration saliva was used, the saliva was wet at the time of contact and the contact involved friction. The five samples where detectable DNA quantities were yielded from fabric samples also generated LRs that provide extremely strong support for the inclusion of the saliva donor. Therefore, the findings of the highly controlled transfer experiments demonstrate that under certain conditions DNA can be transferred through the surface of a paper evidence bag.

Interestingly, in all but one experiment where DNA was detected on the previously DNA-free cotton, undetected quantities of DNA were yielded from the outer surface of the bag. This result may suggest that the tapelift had a greater ability to collect biological material from the cotton compared to the paper. Due to the dislodging of paper fibres on the surface of the bag after contact within the friction set-ups, the adhesiveness of the tapelift was lost faster when sampling the paper compared to the fabric. This rapid loss in adhesiveness may have resulted in the collection of less material from the surface of the paper evidence bag. In addition to visual shedding of fibres from the bag, friction contacts also appeared to have a thinning effect on the surface of the bag, which may be responsible for the apparent increased permeability of the bag.

The difference in the structure of the fibres within the cotton and paper may also explain the difference in the amounts of DNA detected. Due to the naturally hydrophilic cellulose fibres, cotton is good at retaining liquids but poor at releasing them [27, 28]. While both cotton and paper consist of cellulose, hydrophobic additives are often used in papermaking to alter water absorption [29, 30]. These modifications make certain types of paper less hydrophilic than untreated cotton. This was reinforced within this study with the observation that the saliva was absorbed by the cotton significantly quicker than the paper. Due to the more absorbent nature of the cotton, the saliva may

have been wicked away from the surface of the paper, which would explain why less DNA was detected on the outer paper surfaces.

Transfer of DNA through the surface of the bag may explain the DNA observed within the samples that yielded an undetected DNA quantity and generated a low inclusionary LR to the saliva donor but does not explain the samples that yielded an LR which supported the exclusion of the saliva donor. The alleles within some of these profiles could be attributed to the sampler, however the most of these samples contained DNA from an unknown source. The additional source of DNA within the samples, where the profile was assigned a NoC of 2 was also unknown. DNA collected from the gloves of the sampler, while handling the tapelift to put it into the extraction tube may explain the presence of this additional DNA [31].

In the experiments where transfer between bags was tested, it was demonstrated that DNA can transfer between the surfaces of paper evidence bags when the contact involves movement. The DNA quantities yielded from the surface of the paper bag in direct contact with the dosed bag were higher in experiments where friction and movement were applied, in comparison to the experiments where passive contact and pressure was applied. These results align with the observations from the experiments where transfer through the bag was tested and the findings within previous studies [12, 13, 32].

The difference in the DNA quantities between the dosed and transfer paper was most pronounced in the passive contacts, which supports that more DNA was retained by the dosed paper and less DNA was transferred from the dosed paper to the transfer paper. In comparison, the less pronounced difference in the quantities of DNA recovered from each sample type within the friction setups support that more material was transferred to the transfer paper and less was retained by the dosed substrate. The observation of similar interaction patterns in movement and pressure contacts, indicate that while contact type impacts the amount of DNA retained on the dosed bag surface, it does not necessarily translate to equivalent DNA transfer. One explanation for the lower amounts of DNA detected on the dosed paper within the pressure setups, without any apparent DNA transfer to the transfer paper, is that the added weight forced the cellular material into the fibres of the paper on to which the saliva was deposited. The effect of the weight within the friction setups may have been less pronounced due to the movement resulting in the material being dislodged from the bag fibres.

Interestingly, no DNA was yielded in the samples taken from the outer surfaces of either exhibit bags within the friction setups. Due to the porous nature of the paper evidence bag, it was expected that some DNA may be detected on the underside of the paper on to which the saliva was directly deposited, however this was not observed. It is possible that some DNA did permeate through the surface of the bag but was not detected on the surface of the bag. This result would be consistent with the results from the experiments where transfer through the bag was tested. While

the glass on the other side of the substrate was not sampled, it is possible that some of the deposited saliva did permeate the bag and accumulate on the glass on the other side of the paper.

The DNA profiling results from these experiments further reinforced the quantitative DNA findings. All samples taken from the dosed paper yielded an LR which provided extremely strong support for the inclusion of the saliva donor as a profile contributor. Given that the saliva from this individual was deposited directly onto this surface, this was the expected result. In the experiments where passive and pressure setups were tested, only two DNA profiles were generated from the samples taken from the transfer paper. One of these samples yielded a low inclusionary LR to the saliva donor and the other yielded an LR which favoured exclusion of this individual. Another sample taken from the transfer paper within an experiment where movement was tested yielded an LR that favoured exclusion of the saliva donor. All other LRs yielded from the transfer paper in experiments where movement was applied ranged from moderate to extremely strong support for the inclusion of the saliva donor as a profile contributor. The LRs yielded from the transfer paper in experiments where friction was applied ranged from strong to extremely strong support for the saliva donor as a profile contributor. The sources of the DNA within the two samples that yielded LRs that favoured exclusion of the saliva donor were unknown.

While less DNA is known to transfer in contacts which involve porous substrates, dried biological materials and passive or pressure contacts [12, 13], with the magnitude of the DNA deposited onto the dosed paper, it was expected there would be some level of detectable DNA transfer observed between the paper dosed with saliva and the paper that had been in direct contact. Apart from a single instance where a sample from the transfer paper yielded a low inclusionary LR to the saliva donor, this was not observed in experiments that involved passive or pressure contact. Additionally, there were four samples within the movement setups and two samples within the friction setups where no alleles were observed. These results may provide further support that the paper bag surface effectively retains DNA or that biological material is not able to be recovered from the exhibit bag surface or tapelift particularly well. In previous studies, where the same tapelifts and extraction methodologies were implemented, poor recovery of DNA is not obvious from the quantities of DNA recovered from paper evidence bags tapelifted [8, 9]. However, the amount of DNA initially present on the bags sampled within these studies was unknown, so the true efficiency of the sampling is also unknown [8, 9].

4.2.5.2 Part 2- Semi-realistic DNA transfer experiments

Within the highly controlled transfer experiments, it was observed that transfer between the exterior surface of bags occurred under conditions that involved friction and movement, and transfer through the bag occurred within experiments where friction was combined with wet conditions. The semi-realistic experiments explored whether such transfer occurs in practice during processes that mimic realistic handling, storage and transport of exhibits.

In the semi-realistic experiments where DNA transfer through the bag was tested, it was expected that DNA from the wet, sweaty t-shirt, may permeate the surface of the bag during contact between the bag and the plastic tub. From the highly controlled experiments it was expected that if DNA transfer through the bag was observed, DNA would likely be detected on surfaces in direct contact with the outer surface of the bag and potentially on the outside surface of the bag itself.

The only samples within these experiments that yielded quantifiable DNA amounts were taken from the interior surface of the tub. The presence of DNA in the samples taken from the tub before use suggests that the cleaning process did not effectively remove all the DNA. While DNA was present within the tubs before use, DNA from the t-shirt donor was not detected in any samples taken from the storage tubs before use. The slight increase in the quantities of DNA within samples taken from the tub after the movement phase and again following a 2 hour storage period indicates that during these processes DNA was added to the tub. In replicates 1 and 2, the LRs yielded from the samples taken from the tub after the storage period provided slight support for the inclusion of the shirt donor. This result indicates that during the storage period, DNA from the t-shirt may have permeated the surface of the bag and transferred to the plastic tub. The ability for DNA to be detected on the plastic tub, a surface in direct contact with the outer surface of the bag, without the detection of DNA on the bag itself was similarly demonstrated within the highly controlled experiments.

In replicate 2, the sample taken from the exhibit bag before use also yielded an LR that provided slight support for the inclusion of the t-shirt donor as a contributor to the profile. As the t-shirt had not been handled prior to this sample being taken, it is considered unlikely that DNA was transferred from the t-shirt to the bag during the experimental setup. The bags used within experiments are not DNA-free, therefore it was expected that DNA may be present on the bags before use. The inclusionary LR generated between the sample taken from the bag before use and the t-shirt donor could be the result of an adventitious match between DNA that was already present on the bag as a result of the manufacturing process. All following samples taken from the same exhibit bag yielded LRs that provided support for the exclusion of the t-shirt donor as a profile contributor, which indicates that this DNA was removed from the sampling area of the bag during the sampling process. The only other sample taken within these experiments that yielded a low LR to the t-shirt donor was taken from the bag after movement. This may indicate that during the movement of the exhibit in this replicate, DNA permeated the surface of the exhibit bag.

While these results indicate that DNA transfer through the bag may have occurred, with the magnitude of DNA quantities and LRs generated between samples and the t-shirt donor, these results may also be able to be explained by the presence of DNA from other unknown sources. DNA that was present on an unsampled surface of the plastic tub or bag or on the packing tape that is not DNA-free may explain the sources of additional DNA detected within these experiments.

DNA transferred onto the tapelift by the samplers gloves while putting it into the extraction tube could also account for additional DNA [31].

Despite the exhibits inside the bags being wet with a source of DNA and subjected to movement and friction, low DNA quantities and LRs to the t-shirt donor were yielded by samples within these experiments. These results suggest that, if DNA transfer occurred during the simulated handling within these experiments, the risk of significant DNA contamination via the penetration of DNA through the surface of a paper evidence bag appears low.

In the semi-realistic experiments where the transfer of DNA between the surfaces of evidence bags was investigated, it was expected that DNA from the individual who had handled the outside surface of an exhibit bag would be transferred to the surface of the exhibit bag during handling and to the surface of the other exhibit bag during the contact which involved movement. Cellular material deposited by touch is known to contain less DNA than biological fluids [22, 32, 33]. Therefore, it was expected that lower quantities of DNA would be observed on the evidence bags within the semi-realistic experiments where touch DNA was deposited onto bags, compared to the controlled experiments, where saliva was deposited onto bags. Previous studies indicate that a significant portion of the DNA which accumulates on the exterior of exhibit packaging is deposited onto the bag during contact by individuals who handle the exterior packaging of exhibits [9]. It is therefore expected that touch DNA was likely to be a more realistic representation of DNA accumulating on the exterior of exhibit packaging compared to saliva.

Previous studies demonstrate the potential for an individual to transfer their DNA to a substrate via direct contact [34-37], therefore it was expected that DNA from the bag handler would be deposited onto all bags during contact. In two of three replicates, the evidence bags that had been directly handled by the DNA donor yielded detectable levels of DNA and LRs with extremely strong support for the inclusion of the bag handler as the source of DNA after contact with the other exhibit bag. In the third replicate, the sample taken from the handled bag after contact yielded an undetected DNA quantity and produced an LR favouring exclusion of the DNA donor. Only one other sample within this replicate yielded a detectable amount of DNA and the LR generated favoured the exclusion of the DNA donor as a contributor to the profile. This result suggests that a lower amount of DNA may have been deposited onto this bag during handling, compared to the bags within the other replicates. Variability in the amount of DNA deposited by an individual has been previously demonstrated within the literature [38, 39].

Only one sample taken from the bag that was in direct contact with the DNA loaded exhibit package yielded an LR that favoured inclusion of the bag handler. In this replicate, a sample taken from the unhandled bag before contact generated a low inclusionary LR to the DNA donor. With the magnitude of the LR, this match could potentially also be explained by an adventitious match to DNA that was already present on the bag.

Within these experiments, the undetected DNA quantities yielded in samples taken from the tub before use suggests that the cleaning process was effective. In two replicates, the plastic tub surfaces yielded undetected quantities of DNA following contact with the evidence bags with LRs generated that supported exclusion of the bag handler as the source of the DNA. The sources of DNA within these samples were unknown, but could be explained by the presence of DNA transferred from the materials used in the experiments that were not DNA free, which included the evidence bags and packing tape.

In addition to the negligible DNA quantities and low LRs for the DNA donor, inconsistent results were observed across the replicates within these experiments. These findings suggest that within the exhibit handling and transport scenarios in these experiments, the level of DNA transfer between surfaces of a paper evidence bag appeared low.

4.2.5.3 Overall findings, limitations and forensic implications

Low levels of DNA were detected in samples taken within the semi-realistic experiments where DNA transfer through the bag was tested, and substrates tested for transfer generally yielded very low inclusionary LRs to the DNA donors. The magnitude of these LRs suggests that some of the DNA detected could have been present as a result of an adventitious match with background DNA, rather than an indication of transfer of DNA from an exhibit. These findings contrast with those observed in the highly controlled experiments, where friction and a wet source of DNA facilitated transfer through the bag surface. This discrepancy reinforces the variable nature of results observed within DNA transfer research and highlights the difference in impacts that even contacts of a similar nature can have on the amount of DNA transferred.

Experimental findings suggest that during the handling of exhibits throughout the forensic process, unless substantial friction and wet biological material is present on an exhibit, the risk of transfer of this DNA through a paper evidence bag is low. Therefore, compared to dry exhibits, exhibits which are packaged while wet are likely to pose a greater risk of transfer through the exhibit packaging. If the DNA detected on the exterior of the bag in semi-controlled experiments was considered to originate from the exhibit inside the bag, these findings indicate the quantities of DNA transferred between an exhibit and the exterior of the bag are so low that the risk of subsequent transfer of the material resulting in detectable contamination is minimal.

The results of experiments where DNA transfer between bags was investigated indicates that if no movement was involved in the contact, paper is a poor mediator of DNA transfer. Data clearly shows that DNA remains predominantly on the originally dosed paper, and that transfer to other paper substrates is minimal unless movement, particularly friction, is involved. Even then, the DNA transfer observed is inconsistent and under realistic conditions of handling an exhibit performed within this study, was often insufficient to yield detectable levels of DNA or LRs which provide strong support for inclusion of the DNA donor.

While findings of this study indicate that DNA transfer between the surfaces of exhibit packaging and other substrates is low, the accumulation of DNA from an exhibit on the exterior of exhibit packaging should be minimised as much as practically possible. Unlike the detection of DNA from forensic staff on an exhibit, which can usually be identified through comparison with a laboratory elimination database, DNA transferred from one exhibit to another is more difficult to detect and should be avoided.

While this study aimed to replicate realistic conditions, some limitations must be acknowledged. It is probably not possible to perform semi-realistic experiments which will fully reflect the variable and complex dynamics present in operational exhibit processing. For example, in some cases transport time will be significantly longer and heavier exhibits are likely to be subjected to far more friction than was tested within these experiments. In addition, a single biological fluid and substrate were used for mock exhibits, which may not accurately capture the variability encountered in actual casework involving other fluids or materials.

If DNA transfer from a non-porous substrate, or other types of fabric was tested within this study instead, the observed rates of transfer would likely vary. Cotton is a commonly encountered fabric that has been previously used within numerous studies where DNA transfer has been investigated. When compared with non-porous substrates, DNA is more likely to be retained and less likely to be transferred from cotton and other porous substrates. Given that other studies have demonstrated that non-porous substrates transfer DNA more readily than porous substrates, the effect of DNA transfer through the surface of the bag may be amplified with exhibits of a non-porous nature.

With respect to transfer, it has previously been demonstrated that blood, saliva and pure DNA behaved similarly, but skin cells behave differently. It is therefore expected that exhibits on which other biological fluids are present may exhibit similar results to saliva within this study, but less transfer will be observed between the exhibit bag and exhibits that contain touch DNA.

The amount of saliva deposited within the highly controlled experiments is also not likely to be a realistic representation of the amount of biological fluid that may be present on some exhibits, such as a heavily bloodstained piece of clothing. With more heavily stained items, there may be an increased risk of transfer than was observed within these experiments.

When combined, each transfer demonstrated within Part 1 of this study indicates that in contacts that involve friction, it is possible that DNA could be transferred from a wet exhibit, through the surface of a paper evidence bag, onto the surface of another exhibit bag, through another exhibit bag and onto a secondary exhibit. While it was demonstrated within the highly controlled experiments that DNA could be transferred through the surface of a bag and onto another substrate, a significant amount of DNA would need to be present on the exhibit for it to migrate through the bag and still have enough DNA present for an additional transfer. If a scenario is

considered where DNA from a wet exhibit is transferred through the surface of the bag and onto the exterior of another exhibit bag through contact involving friction, another transfer step is required to for the DNA to migrate onto the surface of another exhibit. Whether the final transfer step occurs through the migration of DNA through the surface of the bag, or transfer to another intermediary surface, a significant amount of starting material would still need to be present for this transfer to be detectable on the secondary exhibit.

Findings from the highly controlled experiments can be used to extrapolate approximately how much DNA would need to be present on a wet exhibit for DNA to transfer through the evidence bag (bag 1), transfer to the exterior of another evidence bag (bag 2), permeate the surface of the evidence bag (bag 2) and be detected on the exhibit within. The highest proportion of the initially deposited DNA that transferred through an exhibit bag was 0.014%. The maximum proportion of DNA transferred between the surfaces of paper evidence bags was 0.09%. The smallest quantity of DNA that yielded an LR to the saliva donor greater than 1000 (very strong support for the inclusion of the donor) in these experiments was 0.013 ng. The DNA profile generated from this sample contained 17 alleles and yielded an LR of 1.83×10^6 to the saliva donor. For this DNA quantity to be detected on an exhibit, as a result of DNA transferred through the evidence bag, it is estimated that 92 ng of DNA would need to be present on the exterior of that bag. For this amount of DNA to be transferred during contact with another evidence bag, approximately 100 μg is required on the primary bag. Extrapolating this further, it is estimated that the original exhibit inside of the primary bag would need to contain 7054 μg of DNA for transfer through the bag to explain the detected amount. Assuming human blood contains 30-60 μg/ml of DNA [40, 41], approximately 117- 235 ml of blood would be required to deposit such quantity of DNA. In practice, whether such small quantities of DNA detected on an exhibit from such transfers may be observed will depend on a number of factors, including laboratory quantification thresholds for a sample to proceed for further DNA analysis and analytical thresholds.

The permeability of paper evidence bags to DNA from wet items, and demonstration of transfer between DNA on multiple paper evidence bags, emphasises the need for careful handling and transport of forensic exhibits. When combined with the findings of previous studies, this research demonstrates the potential for cross contamination between exhibits to occur at each stage of the exhibit handling, transport and storage process between the crime scene and forensic laboratory.

These findings contribute to a greater understanding of one potential mechanism behind incidental exhibit contamination. They also prompt laboratories to reassess current exhibit processing procedures, particularly in light of the increasing sensitivity of modern DNA technologies. To mitigate the risk of cross-contamination, strategies such as double-bagging exhibits could be beneficial. Notably, within this study, data suggests that exhibits that are packaged while wet present a heightened risk for DNA transfer compared to those packaged when dry. Where it is

necessary to collect a wet exhibit and transfer it to another location to be dried, caution should be taken if using a single brown paper bag. In general, caution should be taken when handling, storing and transporting wet exhibits. Due to the likely visible staining, it may be apparent if blood has been transferred through the surface of an exhibit bag, while the transfer of other biological fluids such as saliva and sweat may be more likely to go unnoticed due to their lack of visible colour.

Careful management of how exhibit bags are stored and transported could further reduce the risk of DNA transfer between bags and help limit the transfer of DNA from the interior of one bag to the exterior surfaces of another. Where practically possible, minimising contact between exhibit packaging during transport and storage may effectively prevent cross-contamination. This would reduce friction between exhibits and may help to reduce DNA transfer between the packaging of different exhibits. The detection of low-level transfer events, even if infrequent, emphasises the importance of minimising contact between packaging and maintaining separation of exhibits during transportation and storage where possible.

Future research could expand on findings within this study by performing similar experiments which incorporate additional variables, such as a wider range of exhibits and biological fluids, and longer storage durations. Studies exploring the effectiveness of mitigation strategies, such as double bagging or interim containment for wet exhibits would also provide valuable operational guidance.

In summary, the combined findings from this research and previous studies where DNA transfer was observed between exhibit packaging demonstrates the possibility for DNA cross-contamination throughout the exhibit handling, transport, and storage processes, from crime scenes to forensic laboratories. This research assists with providing a better understanding of the mechanisms which may be responsible for incidental exhibit contamination and gives insight into whether current exhibit handling and processing procedures are still adequate given the increased sensitivity of modern DNA technologies. This highlights the need for ongoing assessment and improvement of protocols to ensure the integrity of DNA evidence in forensic investigations.

4.2.6 Conclusions

The findings within this research show that DNA can transfer between paper evidence bags and other porous substrates in secondary contacts. As demonstrated by other studies, the amount of DNA transferred is impacted by contact type, whether the source of DNA is wet or dry and the amount of DNA initially deposited. This study reinforces the principle that DNA transfer through paper evidence bags is possible under specific conditions particularly in the presence of moisture and friction. The risk of transferring DNA between the exterior surfaces of exhibit packaging is also demonstrated, particularly in contact that involve friction. These results highlight the importance of careful consideration by forensic laboratories of how exhibits, particularly those wet with biological material are being handled, transported and stored.

4.2.7 Acknowledgements

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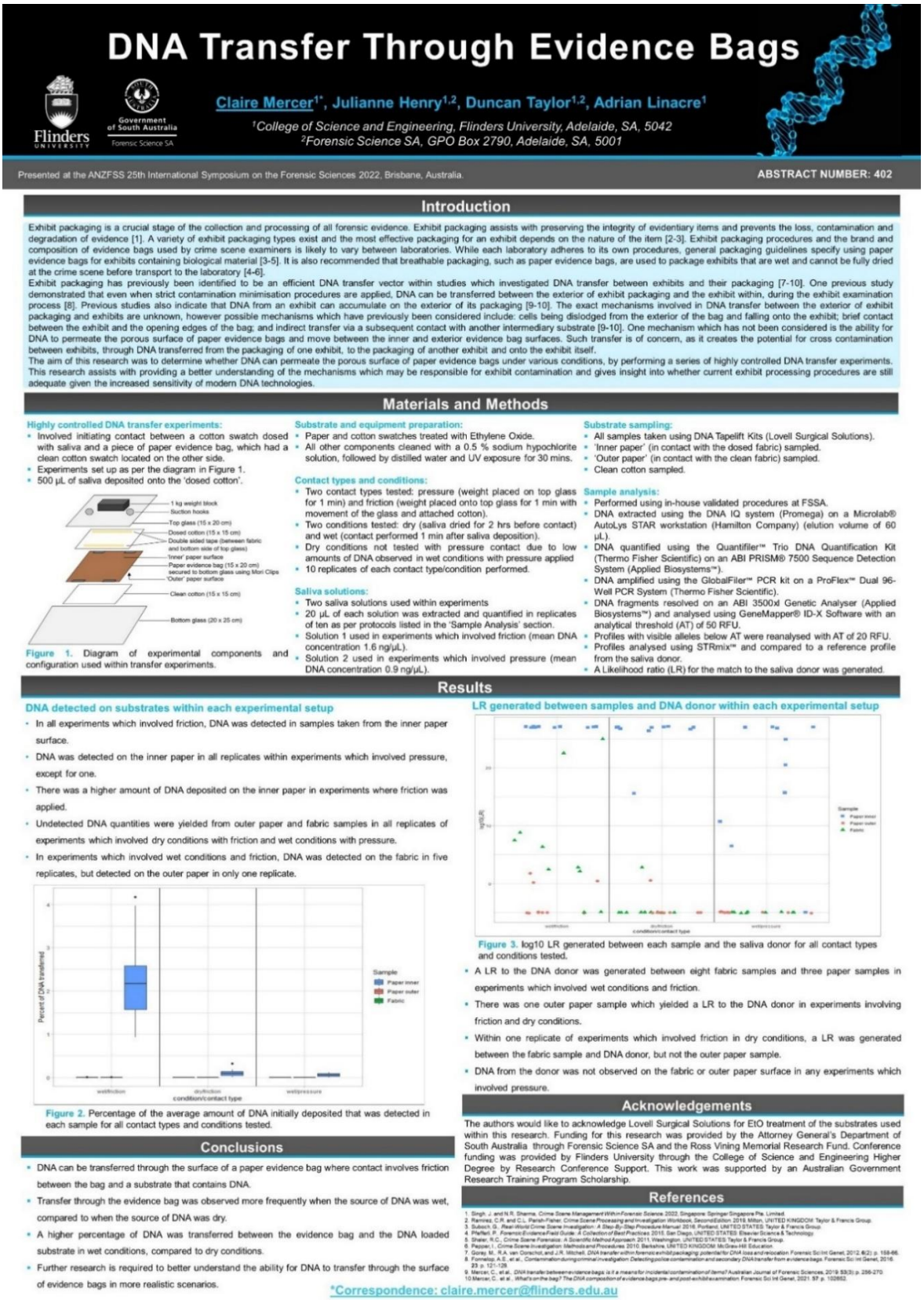
4.2.9 Poster presentation

The following poster was produced from a portion of the data included within this chapter and was presented at the ***25th International Symposium of The Australian and New Zealand Forensic Science Society***, Brisbane, Australia 2022. *DNA Transfer Through Evidence Bags.*



DNA Transfer Through Evidence Bags

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Introduction

Exhibit packaging is a crucial stage of the collection and processing of all forensic evidence. Exhibit packaging assists with preserving the integrity of evidentiary items and prevents the loss, contamination and degradation of evidence [1]. A variety of exhibit packaging types exist and the most effective packaging for an exhibit depends on the nature of the item [2-3]. Exhibit packaging procedures and the brand and composition of evidence bags used by crime scene examiners is likely to vary between laboratories. While each laboratory adheres to its own procedures, general packaging guidelines specify using paper evidence bags for exhibits containing biological material [3-5]. It is also recommended that breathable packaging, such as paper evidence bags, are used to package exhibits that are wet and cannot be fully dried at the crime scene before transport to the laboratory [4-6].

Exhibit packaging has previously been identified to be an efficient DNA transfer vector within studies which investigated DNA transfer between exhibits and their packaging [7-10]. One previous study demonstrated that even when strict contamination minimisation procedures are applied, DNA can be transferred between the exterior of exhibit packaging and the exhibit within, during the exhibit examination process [8]. Previous studies also indicate that DNA from an exhibit can accumulate on the exterior of its packaging [9-10]. The exact mechanisms involved in DNA transfer between the exterior of exhibit packaging and exhibits are unknown, however possible mechanisms which have previously been considered include: cells being dislodged from the exterior of the bag and falling onto the exhibit; brief contact between the exhibit and the opening edges of the bag; and indirect transfer via a subsequent contact with another intermediary substrate [9-10]. One mechanism which has not been considered is the ability for DNA to permeate the porous surface of paper evidence bags and move between the inner and exterior evidence bag surfaces. Such transfer is of concern, as it creates the potential for cross contamination between exhibits, through DNA transferred from the packaging of one exhibit, to the packaging of another exhibit and onto the exhibit itself.

The aim of this research was to determine whether DNA can permeate the porous surface of paper evidence bags under various conditions, by performing a series of highly controlled DNA transfer experiments. This research assists with providing a better understanding of the mechanisms which may be responsible for exhibit contamination and gives insight into whether current exhibit processing procedures are still adequate given the increased sensitivity of modern DNA technologies.

Materials and Methods

Highly controlled DNA transfer experiments:

- Involved initiating contact between a cotton swatch dosed with saliva and a piece of paper evidence bag, which had a clean cotton swatch located on the other side.
- Experiments set up as per the diagram in Figure 1.
- 500 µL of saliva deposited onto the 'dosed cotton'.

Figure 1. Diagram of experimental components and configuration used within transfer experiments.

Substrate and equipment preparation:

- Paper and cotton swatches treated with Ethylene Oxide.
- All other components cleaned with a 0.5 % sodium hypochlorite solution, followed by distilled water and UV exposure for 30 mins.

Contact types and conditions:

- Two contact types tested: pressure (weight placed on top glass for 1 min) and friction (weight placed onto top glass for 1 min with movement of the glass and attached cotton).
- Two conditions tested: dry (saliva dried for 2 hrs before contact) and wet (contact performed 1 min after saliva deposition).
- Dry conditions not tested with pressure contact due to low amounts of DNA observed in wet conditions with pressure applied
- 10 replicates of each contact type/condition performed.

Saliva solutions:

- Two saliva solutions used within experiments
- 20 µL of each solution was extracted and quantified in replicates of ten as per protocols listed in the 'Sample Analysis' section.
- Solution 1 used in experiments which involved friction (mean DNA concentration 1.6 ng/µL).
- Solution 2 used in experiments which involved pressure (mean DNA concentration 0.9 ng/µL).

Substrate sampling:

- All samples taken using DNA Tapelift Kits (Lovell Surgical Solutions).
- 'Inner paper' (in contact with the dosed fabric) sampled.
- 'Outer paper' (in contact with the clean fabric) sampled.
- Clean cotton sampled.

Sample analysis:

- Performed using in-house validated procedures at FSSA.
- DNA extracted using the DNA IQ system (Promega) on a Microlab® AutoLys STAR workstation (Hamilton Company) (elution volume of 60 µL).
- DNA quantified using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific) on an ABI PRISM® 7500 Sequence Detection System (Applied Biosystems™).
- DNA amplified using the GlobalFiler™ PCR kit on a ProFlex™ Dual 96-Well PCR System (Thermo Fisher Scientific).
- DNA fragments resolved on an ABI 3500xl Genetic Analyser (Applied Biosystems™) and analysed using GeneMapper® ID-X Software with an analytical threshold (AT) of 50 RFU.
- Profiles with visible alleles below AT were reanalysed with AT of 20 RFU.
- Profiles analysed using STRmix™ and compared to a reference profile from the saliva donor.
- A Likelihood ratio (LR) for the match to the saliva donor was generated.

Results

DNA detected on substrates within each experimental setup

- In all experiments which involved friction, DNA was detected in samples taken from the inner paper surface.
- DNA was detected on the inner paper in all replicates within experiments which involved pressure, except for one.
- There was a higher amount of DNA deposited on the inner paper in experiments where friction was applied.
- Undetected DNA quantities were yielded from outer paper and fabric samples in all replicates of experiments which involved dry conditions with friction and wet conditions with pressure.
- In experiments which involved wet conditions and friction, DNA was detected on the fabric in five replicates, but detected on the outer paper in only one replicate.

Figure 2. Percentage of the average amount of DNA initially deposited that was detected in each sample for all contact types and conditions tested.

LR generated between samples and DNA donor within each experimental setup

Figure 3. log10 LR generated between each sample and the saliva donor for all contact types and conditions tested.

- A LR to the DNA donor was generated between eight fabric samples and three paper samples in experiments which involved wet conditions and friction.
- There was one outer paper sample which yielded a LR to the DNA donor in experiments involving friction and dry conditions.
- Within one replicate of experiments which involved friction in dry conditions, a LR was generated between the fabric sample and DNA donor, but not the outer paper sample.
- DNA from the donor was not observed on the fabric or outer paper surface in any experiments which involved pressure.

Conclusions

- DNA can be transferred through the surface of a paper evidence bag where contact involves friction between the bag and a substrate that contains DNA.
- Transfer through the evidence bag was observed more frequently when the source of DNA was wet, compared to when the source of DNA was dry.
- A higher percentage of DNA was transferred between the evidence bag and the DNA loaded substrate in wet conditions, compared to dry conditions.
- Further research is required to better understand the ability for DNA to transfer through the surface of evidence bags in more realistic scenarios.

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CHAPTER 5 : CONCLUSIONS AND IMPACT

5.1 Preface

Within this chapter, the findings of all preceding thesis chapters are summarised and the significant new knowledge this research has added to the forensic community is emphasised. The impact of this research for operational forensic laboratories and the general forensic community are discussed with possible future directives as a result of this work outlined.

Advancements in DNA profiling technologies have significantly increased the sensitivity of DNA analyses. While this increased sensitivity offers improved detection of trace DNA, it also introduces challenges. The ability to detect smaller amounts of DNA increases the likelihood of detecting background DNA that is unrelated to an alleged offence or DNA inadvertently introduced through contamination.

It is well-established that DNA contamination can have severe consequences, which includes the potential for miscarriages of justice. Although stringent procedures are in place within forensic laboratories and during crime scene examinations to mitigate and detect contamination, it is widely accepted that contamination should be prevented wherever possible. Crucially, without a comprehensive understanding of the mechanisms by which contamination occurs, it cannot be effectively prevented.

There is a considerable amount of research that studies sample or exhibit contamination at crime scenes and within laboratories. These studies are related to improper cleaning and the use of contaminated laboratory equipment and consumables, such as examination tools and gloves. However, prior to the work within this thesis, little attention had been given to contamination risks associated with exhibit packaging. The mechanisms by which DNA may be transferred via exhibit packaging and the extent of contamination risk this posed remained largely unknown.

This thesis aimed to address this gap in the literature by providing a deeper understanding of the role exhibit packaging plays in the accumulation and transfer of DNA. It explores how contamination can occur as an exhibit moves through various stages of the forensic process, offering insight into crucial and previously understudied mechanisms of DNA transfer and their implications for the integrity of forensic evidence.

5.2 Chapter Summary

The focus of Chapter 2 was to explore the potential for evidence bags to act as DNA transfer vectors. Evidence bags from casework exhibits were examined at each stage of the exhibit examination process; this was by examining the DNA quantity and composition on the exterior of evidence bags before and after the exhibit examination process.

The magnitude of DNA quantities detected on the exterior of the evidence bags sampled within this study reinforce the realistic opportunity for exhibit contamination that is posed by contact with exhibit packaging. Bi-directional DNA transfer from such contacts has the potential to result in compromised DNA profiles. DNA from individuals who were known to handle the bag was detected on the exterior of the packaging, in addition to individuals who had not handled the bag according to the chain of custody, but worked within areas exhibits were processed and stored. However, much of the DNA detected on the outside surface of exhibit packages could not be attributed to individuals on the laboratory elimination database or the exhibit inside of the bag. In addition to raising the question of the source of DNA, this result reinforces the need for comprehensive laboratory elimination databases that contain DNA profiles from all individuals who not only handle exhibit packaging, but work within areas where exhibits are processed.

Out of the 60 exhibits sampled within this study, there was only a single instance where the potential for DNA transfer from the outer exhibit bag to the exhibit was observed. This transfer could have been explained by two separate primary transfer events rather than a primary transfer to the bag and then secondary transfer to the exhibit. If this instance was interpreted as a true contamination event, that involved sample-to-sample transfer, this represents a rate of approximately 1.7%. This result suggests that, at least for the exhibits and packaging examined within this study, when appropriate forensic protocols are followed, the risk of contaminating evidence via contact with the outer surface of packaging is low.

In 13 instances, DNA from an exhibit was transferred to the outside of its exhibit bag during the examination process. It remains unclear whether these transfers resulted from primary transfer through direct contact between the exhibit and the outside surface of its packing or secondary transfer via the examiner’s gloves; the latter scenario would not involve contamination of the exhibit itself.

This work challenges the commonly held assumption within forensic science that a single brown paper evidence bag is a sufficient barrier to prevent the movement of DNA between the exhibit contained within the bag and the exterior surface of the bag. Under this assumption, the interior of the bag is considered free from external DNA, while the exterior is expected to accumulate environmental DNA from various sources. These findings underlined the importance of evaluating exhibit handling procedures, particularly in light of increasingly sensitive DNA profiling technologies.

This research fills a gap in the literature regarding the potential exhibit contamination risk posed by DNA which accumulates on exhibit packaging and the mechanisms which may be involved in exhibit contamination during the exhibit examination process. The detection of exhibit DNA on the exterior of exhibit packaging also raised critical questions of how far such DNA transfer might extend and whether there is the potential for exhibit DNA to accumulate within forensic

workspaces. These questions informed the direction of the subsequent sections of research within this thesis.

Within Chapter 3, the level of DNA transfer that occurs between exhibit packaging and forensic workspaces was explored, and the levels and sources of DNA that accumulate in forensic workspaces where exhibits are handled and stored were investigated. This study investigated the accumulation and transfer of DNA within a forensic exhibit storeroom over a 14-week period, aiming to assess potential contamination risks in operational forensic environments. Findings show that much of the detectable DNA on the exhibit storeroom shelves was removed at the beginning of the study through cleaning and accumulated again over time.

DNA accumulation was higher in compactus units where exhibits were stored, suggesting that contact between evidence packaging and shelving surfaces was responsible for much of the accumulating DNA. The accumulation of DNA on shelves within empty compactus units, suggests that some level of airborne DNA transfer is also occurring. Staff DNA was commonly detected, with the frequency of detection correlating with how often individuals from specific departments access the storeroom. DNA from police staff, who do not enter the storeroom, was also detected, indicating that indirect transfer is occurring through other intermediaries, such as evidence packaging.

Three instances where DNA profiles from shelf samples matched exhibit DNA profiles were detected, though only one could be attributed to a known staff member match. These matches could have resulted from indirect transfer or adventitious matches due to the high number of comparisons performed. Such findings highlight the risk that DNA from one exhibit could transfer to another via the environment or packaging, potentially leading to cross contamination of exhibits during casework.

Overall, the results indicate that exhibit storerooms can provide an environment where DNA can accumulate and remain for extended periods, creating a source of potentially contaminating DNA. Reducing the amount of environmental DNA within exhibit storerooms may limit the extent that DNA is deposited onto evidence packaging and subsequently reduce the possibility of transfer between exhibit packaging and the exhibit within.

The detection of DNA from both individuals who work within the storeroom and those who do not, reinforces the need for comprehensive laboratory elimination databases that include all personnel who handle exhibits or work within areas they are handled and stored. Although the likelihood of cross-contamination between exhibits within the storeroom was found to be low, the potential implications for forensic integrity underscore the need for continued vigilance and procedural assessment. Several practical recommendations related to the handling, storage, and packaging of forensic exhibits were proposed to further mitigate contamination risk, including routine cleaning of

exhibit storage areas and use of PPE, to minimise the amount of DNA accumulating within forensic workspaces. These measures were not prescriptive and, given the low incidence of contamination observed within this study, it was acknowledged their adoption should be guided by context-specific cost-benefit assessments. While laboratories will always be able to implement more rigorous contamination control procedures, there is a point at which additional interventions offer limited improvements relative to the resources required. It is therefore essential for a laboratory to strike a practical balance between the degree of contamination control and the feasibility of implementing such measures.

Chapter 4 was designed to provide further information around the mechanism behind the accumulation of exhibit DNA on the exterior of exhibit packaging and the potential for evidence bags to act as DNA transfer vectors. Specifically, within this study, the ability for DNA to permeate the porous surface of paper evidence bags and transfer between the surfaces of evidence bags and other surfaces was studied.

It was demonstrated that under certain conditions DNA can transfer between the exterior surfaces of paper evidence bags and permeate the surface of the bag. The amount of DNA transferred within experiments was influenced by the amount of DNA on a substrate, whether the substrate was wet or dry and the nature of the contact. When moisture and friction was present, transfer through the bag was observed. DNA transfer between the surfaces of evidence bags occurred in contacts that involved friction or movement. The ability for such transfer to occur demonstrates the possibility for DNA cross-contamination throughout the exhibit handling, transport, and storage processes, from crime scenes to forensic laboratories. However, unless substantial friction and wet biological material is present on an exhibit, the risk of such transfer resulting in cross contamination between exhibits appears low.

Together, the findings within this thesis highlight the complexity of the level of DNA transfer that occurs between exhibits, exhibit packaging and other surfaces throughout the forensic process. This work further challenges the commonly held assumption that a single brown paper evidence bag is a sufficient to prevent the movement of DNA between the interior and exterior of evidence bags. These results emphasise the importance of the consideration and application of best practice contamination minimisation procedures throughout all stages of the forensic process. To maintain the integrity of evidence and operational efficiency of forensic laboratories, it is necessary to strike a balance between the risk of contamination and the practical limitations of exhibit handling and storage procedures applied throughout the forensic process.

5.3 Future Impacts and Directives

As technologies in forensic science continue to evolve and advance, the sensitivity of DNA technologies and the ability for forensic laboratories to detect very small amounts of DNA is

arguably only likely to improve. The implications of the introduction of these technologies must be considered and if necessary, addressed through procedural refinement by forensic laboratories.

This research contributes to the growing body of evidence that demonstrates that the risk of exhibit contamination is not only present at the crime scene or within the laboratory during exhibit examination, but also can occur via evidence packaging during handling and storage post collection. In forensic science it is well known that contamination can have significant impacts on the outcome of an investigation and the consequences can be extensive and severe. Arguably, the most severe outcome of contamination is the miscarriage of justice. The introduction of contaminating DNA can obscure the presence of crucial evidence by masking the DNA profile of the true perpetrator or render a sample unusable due to the additional complexity involved in the interpretation. Other downstream consequences include damage to the credibility of a laboratory and the forensic process, which can undermine the reliability of the evidence and expert testimony in court and damage the public trust in the system of forensic science. Contamination can also be costly and place a significant financial burden on forensic laboratories, law enforcement agencies and the judicial system due to wasted resources investigating erroneous leads and additional costs incurred in sample re-analysis where it is possible.

This work provides forensic laboratories with a more comprehensive awareness and understanding of the possible mechanisms which can result in the contamination of exhibits during the routine exhibit handling and storage that occurs throughout the forensic process. Consequently, this research may have implications for forensic accreditation standards, how evidence is presented and interpreted within court and overall public confidence in forensic science. To meet national and international accreditation requirements and ensure the consistency, accuracy and validity of results, Standard Operating Procedures are required within forensic laboratories. The work within this thesis provides the foundation for laboratories to evaluate existing operating procedures to identify and assess the possible contamination risks from transfer mechanisms which may not have been previously considered to be probable but have now been demonstrated to be possible within this research. These procedures include proper evidence handling, contamination prevention measures, including the use of PPE, chain of custody protocols, and controlled environments within forensic laboratories that are accessed in a manner that allows the accumulation of DNA to be minimised. These findings can assist with informing future guidelines and best practices for forensic professionals to follow during evidence collection, transportation, and storage and will assist forensic laboratories with updating existing evidence-handling protocols that mitigate contamination risks and maintain the integrity of forensic evidence from collection through analysis. Strict adherence to protocols, improvement of forensic processes, and constant awareness are crucial to minimising contamination risks and ensuring the reliability of forensic evidence in investigations.

Additionally, within the forensic community, it is becoming more common to consider activity level propositions in the evaluation of forensic biology findings. The ability to perform activity level evaluations is underpinned by data from studies which investigate DNA TPPR. When questions around the mechanisms that may explain the transfer of DNA are presented within court, the availability of research relating to DNA TPPR is fundamental to be able to make accurate evaluations. This work contributes to the existing DNA transfer literature, allowing forensic experts to better understand the ability for DNA transfer between exhibits and their packaging throughout the forensic process, which ultimately results in the ability to evaluate forensic biological findings more comprehensively.

Future directives that could be taken from this research include further investigation into the practicality and effectiveness of procedures that have previously been suggested to potentially mitigate the risk of contamination from the DNA transfer mechanisms studied within this work, such as the double bagging of exhibits. Further research into the mechanisms involved in such transfers using procedures that more closely reflect real world casework exhibits and scenarios or using laboratory specific procedures may additionally provide laboratories with a better representation of contamination risks posed under more realistic scenarios or those that more closely reflect their own procedures.

5.4 Final Statement

Prior to the commencement of the work within this thesis, the risk of the potential for exhibit packaging to act as DNA transfer vector was largely unknown. While previous research has extensively explored contamination from crime scenes, laboratory equipment, laboratory workspaces and PPE, the role of exhibit packaging in the introduction of contaminating DNA was understudied. Research which investigated the ability for DNA to be transferred between forensic exhibits, exhibit packaging and forensic workspaces was also clearly lacking and the mechanisms involved in the inadvertent introduction of biological material onto an exhibit as a result of routine exhibit handling and storage was not well understood. In addition, there were no studies which investigated the potential for DNA to be transferred through the surface of paper evidence bags, from the exterior of an evidence bag and to other evidence bags or forensic workspace surfaces.

This thesis explored the level of DNA accumulating on the exterior of casework exhibit packages, the sources of accumulating DNA and the potential for this DNA to be transferred further to other exhibits and substrates. Each of the experimental sections provide information about the level of such DNA transfer occurring during routine exhibit handling and storage. This research underscores how exhibit packaging and storage environments can become vectors for unintended DNA transfer. When the results from all experiments are collectively considered, insight into the transfer of DNA which may occur between exhibits, packages and workspaces, during the whole exhibit handling process is provided. The work within this thesis addresses this gap within the

literature and provides critical insight into the contamination risks associated with exhibit packaging and contamination risks posed as exhibits move throughout the forensic process. This research contributes to a more comprehensive understanding of contamination minimisation within forensic science.

This research provides evidence-based justification for continuous process improvements, staff training, and practical investments in environmental monitoring and contamination control. All forensic laboratories can utilise this research to inform best practice contamination minimisation procedures. In light of this research, it is recommended that laboratories assess current procedures and re-evaluate their procedures with the future introduction of any new technologies where sensitivity is increased. The challenge remains in striking a realistic balance between contamination risk reduction and operational feasibility, particularly across multiple settings as variable as crime scenes, police stations, exhibit storage facilities and forensic laboratories. Given the potentially severe consequences of contamination in forensic investigations, rigorous preventative measures are crucial.

In conclusion, this research highlights a complex but potentially significant impact that DNA transfer between exhibits and exhibit packaging can have on the integrity of forensic evidence. Understanding and addressing these risks through the improvement of procedures and awareness within the forensic community is essential to preserve the reliability of forensic findings and maintain confidence in forensic science, which is vital to the integrity of the justice system.

CHAPTER 6 : APPENDICES

6.1 Additional Manuscript

The following publication was a result of research conducted immediately prior to the candidature. As the manuscript was prepared for publication during the duration of the candidature and contributes to the overall theme of the thesis, it is added as an appendix for reasons of completeness of the work conducted.

This manuscript was accepted for publication in the Australian Journal of Forensic Sciences, 53(3):256-270. https://doi.org/10.1080/00450618.2019.1699957

6.2 Statement of authorship

Author	Contribution (%)			Signature	Date
	Research Design	Data collection and analysis	Data Interpretation and Evaluation		
Claire Mercer	**70**	**70**	**70**	**Claire Mercer**	**7/11/2023**
Damien Abarno	10	10	10	Damien Abarno	11/12/2023
Phillippa Hearnden	10	10	10	Phillippa Hearnden	11/12/2023
Adrian Linacre	10	10	10	Adrian Linacre	24/10/2023

6.3 Manuscript: DNA Transfer between Evidence Bags: is it a means for incidental contamination of items?

Claire Mercer, Damien Abarno, Phillippa Hearnden, Adrian Linacre. DNA transfer between evidence bags: is it a means for incidental contamination of items? Australian Journal of Forensic Sciences (2021) 53(3): p. 256-270.

6.3.1 Abstract

With increasing sensitivity of DNA profiling systems, the opportunity to detect incidental DNA transferred between evidential items has increased. Evidence bags are assumed to be DNA transfer vectors which can cause inadvertent transfer of DNA between items, yet little has been studied to indicate the extent of this transfer. This study aimed to provide insight into the levels of DNA accumulating on evidence bags through exhibit handling and storage. Casework exhibit bags were sampled during storage and mock exhibit bags were sampled after replicating exhibit handling protocols. DNA concentrations recovered from casework exhibit packages were highly variable and produced profiles containing up to at least seven contributors. The DNA concentration and profile complexity was higher in samples taken from packages after exhibit examination. It was observed that DNA from the exhibit can be transferred to the exterior of the bag during the process of packaging and un-packing the item. Profile complexity from mock exhibit packages increased with time spent in the exhibit storeroom. With the introduction of highly sensitive DNA profiling systems, procedures involving the handling, transport and storage of exhibits should be assessed to ensure that best practice contamination minimisation procedures are being utilised.

6.3.2 Introduction

DNA profiling is becoming increasingly sensitive, and with this benefit comes the opportunity to detect DNA at trace amounts. DNA from higher order transfer mechanisms are being detected more frequently and the chance of detecting DNA within a sample which was unrelated to an alleged incident has also increased[1, 2]. Previous research has demonstrated that DNA can be transferred to a surface via direct contact, and indirectly through one or more transfer vectors [1, 3-6]. This highlights the importance of investigating the transfer of DNA within areas where casework exhibits are handled and stored, and identifying potential transfer vectors.

To minimise the risk of staff DNA contamination, forensic laboratories utilise strict operational procedures, which include the use of Personal Protective Equipment (PPE) and comparison of evidence DNA profiles with a database containing staff DNA profiles. Use of a staff DNA database may identify the source of the contaminating DNA but may not indicate how the event occurred. Previous studies have identified gloves and laboratory tools as efficient DNA transfer vectors, which can cause contaminations during the examination of an exhibit [1, 7-11].

There are limited studies which investigated the potential for evidence bags to act as DNA transfer vectors [12, 13]. At some steps in the handling of evidence bags it might not be standard practise to use gloves, which may result in the accumulation of DNA on the outside of the packages. A recent study identified that even when strict contamination minimisation procedures are followed, transfer of DNA from the outside of an evidence bag to an exhibit inside can occur during the examination process [12]. The results of this study also implied that the risk of exhibit contamination increased with an increase in the concentration of DNA found on the outside of the evidence bag [12, 13].

Currently, there are no studies which provide an insight into the level of DNA which accumulates on the exterior of an exhibit package as it moves from a crime scene to the laboratory. There are also limited studies that investigate the potential for the DNA on the outside of an exhibit package to be transferred to other surfaces [12]. The aim of this study was to determine the DNA load accumulating on evidence bags throughout the handling and storage of exhibits, and provide insight into the potential for evidence bags to act as DNA transfer vectors.

6.3.3 Materials and Methods

This study consisted of three parts, which collectively involved sampling exhibit bags at various stages of handling and storage. In Part 1, casework exhibit packages were sampled at various stages of handling, and in Parts 2 and 3 mock exhibit packages were sampled during experiments which replicated these processes. As the evidence bags sampled in Part 1 contained different casework exhibits, the size of each of the bags varied. The brand of some of these evidence bags may have also varied, as they were received from different police locations, however, all evidence bags sampled were brown paper evidence bags. The evidence bags used in the positive control and Parts 2 and 3 were approximately 30 cm x 40 cm and were supplied by Savill Packaging. Within all experimental parts, control samples were taken in the same manner and from the same location as the experimental samples to maintain consistency.

6.3.3.1 Sampling technique

All samples were taken from the exterior of paper evidence bags using DNA Tapelifts (Lovell Surgical Solutions), which were repeatedly pressed onto the bag's surface until the designated area was sampled. The chosen sampling area for all parts of the study was the top half of the evidence bag, including the evidence tape seal. All samples were taken by individuals wearing PPE.

6.3.3.2 Experimental controls

A positive control was performed by sampling a bag which had been held by two individuals for 10 seconds, 30 minutes post-handwashing. A negative control was produced by placing an unused tapelift into a tube for DNA analysis.

6.3.3.3 Part 1

Samples were taken from 10 randomly chosen casework evidence bags before and after exhibit examination. These samples were taken by members of the Evidence Recovery department at FSSA Biology, who examined the exhibit inside the bags that were sampled as part of routine casework.

6.3.3.4 Part 2

Mock exhibits were produced, while wearing PPE, by placing an unused disposable laboratory coat into a paper evidence bag and sealing the bag with tamper-proof tape. A total of nine mock exhibits, which had an entire size of approximately 30 x 30 cm, were included in this study. To establish a baseline DNA level on the exterior of bags, a sample was taken from the top half of all exhibit packages before placing them into the exhibit storeroom. Three of the exhibits were placed onto each of three shelves within the same compactus. After one week, one exhibit from each shelf was sampled. Another exhibit from each shelf was sampled at four weeks and the three remaining exhibits were sampled after eight weeks. Throughout the period of storage within the storeroom, the mock exhibits were stored with casework exhibits. Contact between the packages of the mock and casework exhibits was observed to occur at various stages of storage and not controlled deliberately, to replicate realistic exhibit storage.

6.3.3.5 Part 3

Two experiments were performed to replicate the packaging of an exhibit at a crime scene, as well as the packaging and removal of an exhibit in the laboratory during examination. The mock exhibit used in these experiments was a cotton T-shirt, which had been worn previously by an individual while running for approximately 30 minutes. Throughout both experiments, a sample was taken from the outside of an evidence bag at four defined stages. The top half of the evidence bag was sampled immediately before it was used in both experiments. In the first experiment, the T-shirt was placed onto the outer surface of the bag for 30 seconds, to replicate the process of photographing an exhibit at a crime scene; which anecdotally has been observed to occasionally occur before the item is packaged. The T-shirt was then placed into the bag, which was sealed using evidence tape, before taking a sample from the outer surface. In the second experiment, the T-shirt was placed directly into the bag, which was sealed using evidence tape, before taking a sample from the outer surface. For both experiments, a sterile disposable scalpel was used to make an incision in one side of the bag, creating a hole large enough to easily remove the item. The T-shirt was removed from the bag by tipping it upside down and the empty bag was tapelifted. The T-shirt was placed back into the bag through the incision, the bag resealed, and then sampled in both experiments. Gloves were changed after taking each sample and before taking samples at every stage which involved directly handling the T-shirt. Both experiments were repeated three times, using a cotton T-shirt from the same individual. The individual who donated the T-shirts

provided informed consent to have their reference DNA profile compared to the profiles generated from samples taken in these experiments.

6.3.3.6 Sample processing

All stages of DNA analysis were performed using in-house validated protocols at FSSA. DNA was extracted from samples using the DNA IQ system (Promega) on a Multiprobe II 8 tip Liquid Handling Platform (LHP) (PerkinElmer), using an elution volume of 60 µL. A Janus® 4 tip LHP (PerkinElmer) was used to prepare purified DNA samples for quantification and samples were quantified using the Quantifiler™ Trio DNA Quantification Kit (Applied Biosystems™) on an ABI PRISM® 7500 Sequence Detection System (Applied Biosystems™). DNA amplification was performed on a ProFlex™ Dual 96-Well PCR System (Thermo Fisher Scientific) using the GlobalFiler™ PCR kit (Applied Biosystems™). Cycling conditions were as per manufacturer's recommendations for 29 cycles with a maximum DNA volume of 15 µL in a final volume of 25 µL. Amplified DNA fragments were resolved using an ABI 3500xl Genetic Analyser (Applied Biosystems™).

6.3.3.7 DNA profile interpretation

The amplified DNA fragments were analysed using GeneMapper® ID-X Software with an allele detection limit of 50 relative fluorescence units (RFU). Profiles with alleles below 50 RFU were reanalysed in GeneMapper® ID-X using an allele detection limit of 20 RFU. For each profile generated, the minimum number of individuals required to reasonably explain the profile was determined.

Profiles generated from the samples taken in Part 3 of the study were analysed using STRmix™ software. It was decided to limit analysis in STRmix™ to profiles containing a maximum of four contributors, partly due to having limited access to computers with the required power to analyse profiles with a higher number of contributors. Profiles which were determined to contain more than four contributors were reanalysed in GeneMapper® ID-X, using an allele detection threshold of 175 RFU. The number of contributors within these profiles were re-assessed and those determined to contain less than four contributors were analysed using STRmix™. The reference DNA profile from the individual who donated the shirt in Part 3 was then compared to analysed profiles. For each comparison a likelihood ratio (LR) was calculated using the propositions:
H1: The T-shirt donor and (N-1) unrelated individuals are the sources of DNA.
H2: N individuals, unrelated to the POI are the sources of DNA.
where N is the number of profile contributors. A theta value was not applied to the LR calculations and the point estimate was used. An in-house GlobalFiler™ Caucasian allele frequency database consisting of 226 individuals was used for LR calculations.

6.3.3.8 Data Analysis

A Wilcoxon Signed-Rank Test was performed using the concentrations of DNA and contributors in profiles from samples taken before and after exhibit examination in Part 1 of the study. A Single-factor Analysis of Variance (ANOVA) test was performed using the concentrations of DNA recovered from the samples taken in Part 2 of the study. In all statistical tests, a significance level of $p<0.05$ was tested.

6.3.4 Results

6.3.4.1 Part 1

Casework exhibit packages were sampled to investigate the DNA load accumulating on packages as they moved through FSSA to the laboratory and determine if DNA was added to or removed from the package during the examination process. The concentrations of DNA recovered from the tapelifts taken from the ten exhibits before and after exhibit examination are given in Figure 6.1.

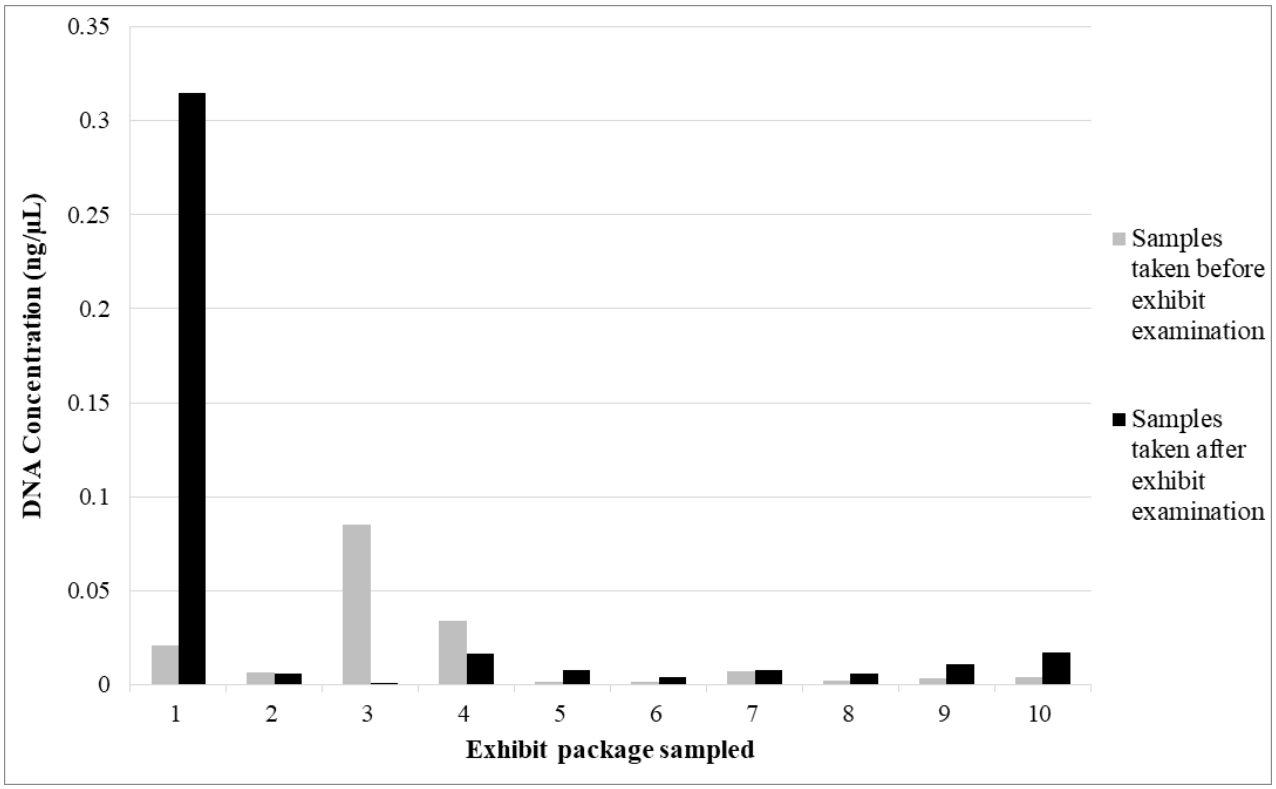

Figure 6.1. The concentration of DNA recovered (ng/µL) from ten exhibit packages tapelifted before (grey) and after (black) the examination of the exhibit inside of the package.

On seven of the ten exhibit bags sampled, the concentration of DNA recovered from the sample taken after exhibit examination was higher than the sample taken before. The concentration of DNA recovered from the sample taken from exhibit 1 after examination was 0.31 ng/µL. This value was higher than all other samples within this part of the project. The highest concentration of DNA recovered from a package before exhibit examination was 0.085 ng/µL and this was from exhibit 3. There was less variation in the concentrations of DNA recovered from the samples taken from the

package before exhibit examination, which ranged from 0.0017- 0.085 ng/μL. In comparison, the concentrations of DNA recovered from samples taken after exhibit examination ranged from 0.0010- 0.31 ng/μL. The results of a Wilcoxon Signed-Rank Test confirmed that there was no statistically significant difference between the concentrations of DNA recovered from the exhibit packages before and after exhibit examination (p=8).

DNA profiles were produced from all 20 tapelifts taken in this part of the study. The number of profile contributors was assessed using a peak detection threshold of 50 RFU and are shown in Figure 6.2.

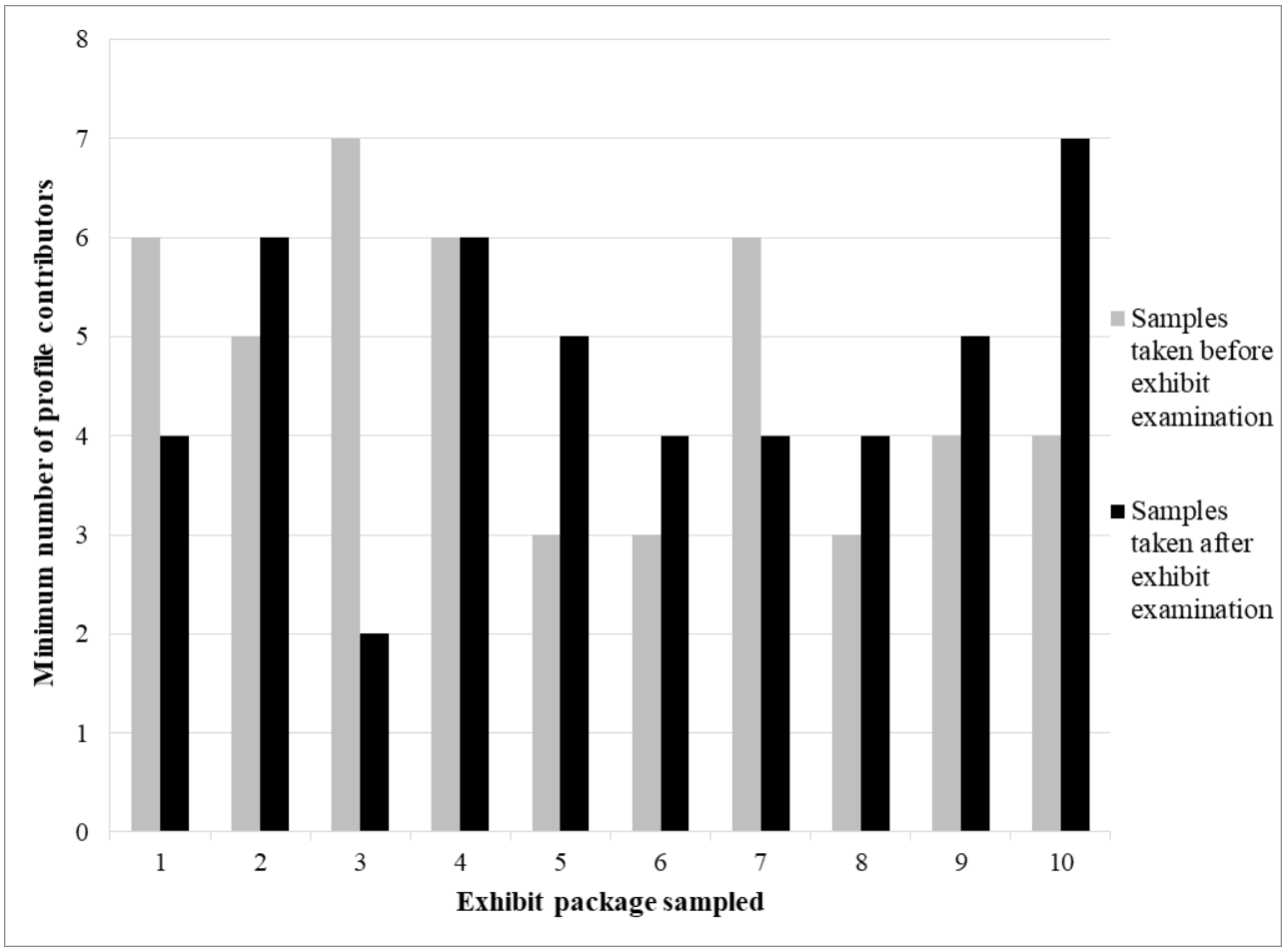

Figure 6.2. The minimum number of contributors in profiles generated from each tapelift taken from 10 exhibit bags before (grey) and after (black) the exhibit inside the package was examined. Contributor numbers assessed using a peak detection threshold of 50 RFU.

From six of the ten exhibit bags tapelifted, the sample taken after exhibit examination produced a profile with a higher minimum number of contributors than the sample taken before. At least 7 contributors were present in the most complex profiles produced from samples taken both before and after exhibit examination. There was less variation in the number of contributors observed within profiles generated from samples taken from the package before exhibit examination, which ranged from 3-7. In comparison, the profile contributors observed within samples taken after exhibit

examination ranged from 2-7. The results of a Wilcoxon Signed-Rank Test confirmed that there was no statistically significant difference between the number of contributors in profiles generated from the exhibit packages before and after exhibit examination (p=3).

6.3.4.2 Part 2

Mock exhibit packages were placed into the FSSA evidence storeroom for different time periods, to determine the DNA load accumulating on evidence bags during storage. The concentrations of DNA recovered from the bags before being placed into the exhibit storeroom and at each time point tested are displayed in Figure 6.1.

Table 6.1. Concentrations of DNA (ng/µL) recovered from tapelifts taken from each mock exhibit bag before they were placed into the exhibit storeroom (baseline) and after 1 week, 4 weeks and 8 weeks of being in the storeroom.

	Concentration of DNA Recovered from sample (ng/µL)					
	baseline	1 week	baseline	4 weeks	baseline	8 weeks
Replicate 1	0.0040	0.0022	0	0.0005	0	0.0042
Replicate 2	0.0006	0.0002	0	0.0024	0	0.0013
Replicate 3	0	0	0.0023	0.0013	0.0003	0.0044

The concentrations of DNA recovered from the samples taken from mock exhibits at different time points were not consistent between samples. In replicates 1 and 3, the highest concentration of DNA was recovered from the bag stored for 8 weeks. In replicate 2, the highest concentration of DNA was recovered from the bag stored for 4 weeks. In all replicates, there were samples where a larger concentration of DNA was recovered from the baseline sample compared to subsequent samples. An ANOVA showed no statistically significant difference between the concentrations of DNA recovered from the bags stored within the exhibit storeroom for 1, 4 and 8 weeks (p=0.13).

At least one DNA profile contributor was observed in all profiles generated, except for two of the baseline samples. The minimum numbers of contributors assessed within all profiles are displayed in Figure 6.3.

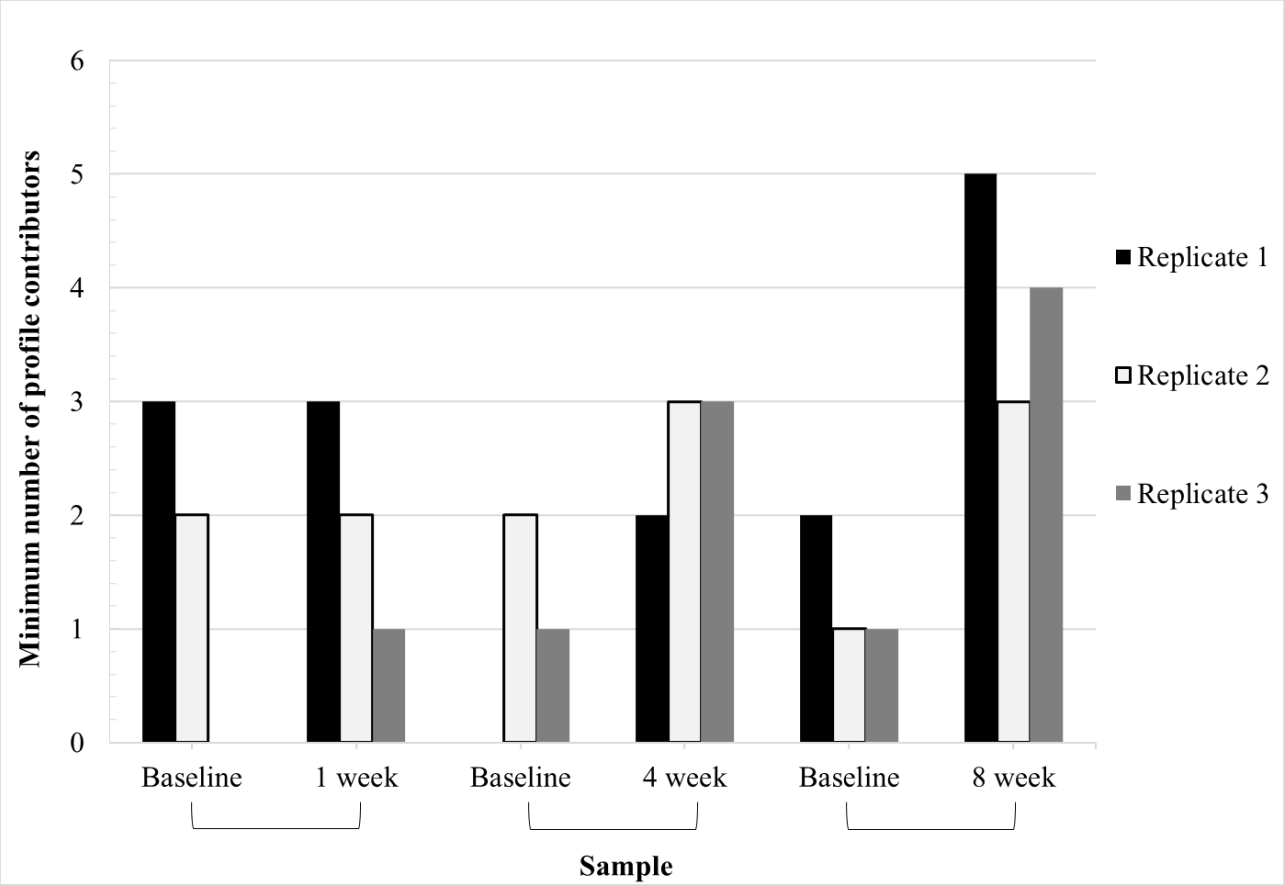

Figure 6.3. The minimum number of profile contributors from mock exhibit packages before being placed into the storeroom (baseline) and 1, 4 and 8 weeks after being placed into the storeroom, for replicate 1 (black), replicate 2 (white) and replicate 3 (grey). The baseline samples taken from the bags are displayed to the left of each of the relevant samples. These profiles were analysed using a peak detection threshold of 20 RFU. As there were no contributors observed in the profiles generated from the baseline values for replicate 3 (1 week) and replicate 1 (4 weeks), these data points are not shown.

Compared to the samples taken at baseline, there was an increase in 0-1 contributors in the profiles generated from samples taken from bags 1 week after storage.
There was an increase in 1-2 profile contributors between the samples taken at baseline and after 4 weeks of storage. The highest numbers of profile contributors were observed in the samples taken after 8 weeks of exhibit storage, with an increase of 2-3 profile contributors compared to the baseline samples.

6.3.4.3 Part 3

This part of the project investigated the potential for DNA transfer to occur between an exhibit and the exterior of its packaging through the process of packaging and removing the item; this was a T-shirt taken from a volunteer after a run. The concentrations of DNA recovered from four samples taken from the exterior of exhibit bags at different stages of two experiments, which both replicated

packaging an exhibit at the crime scene and the removal and insertion of an exhibit during the examination process are displayed in Figure 6.4 and Figure 6.5

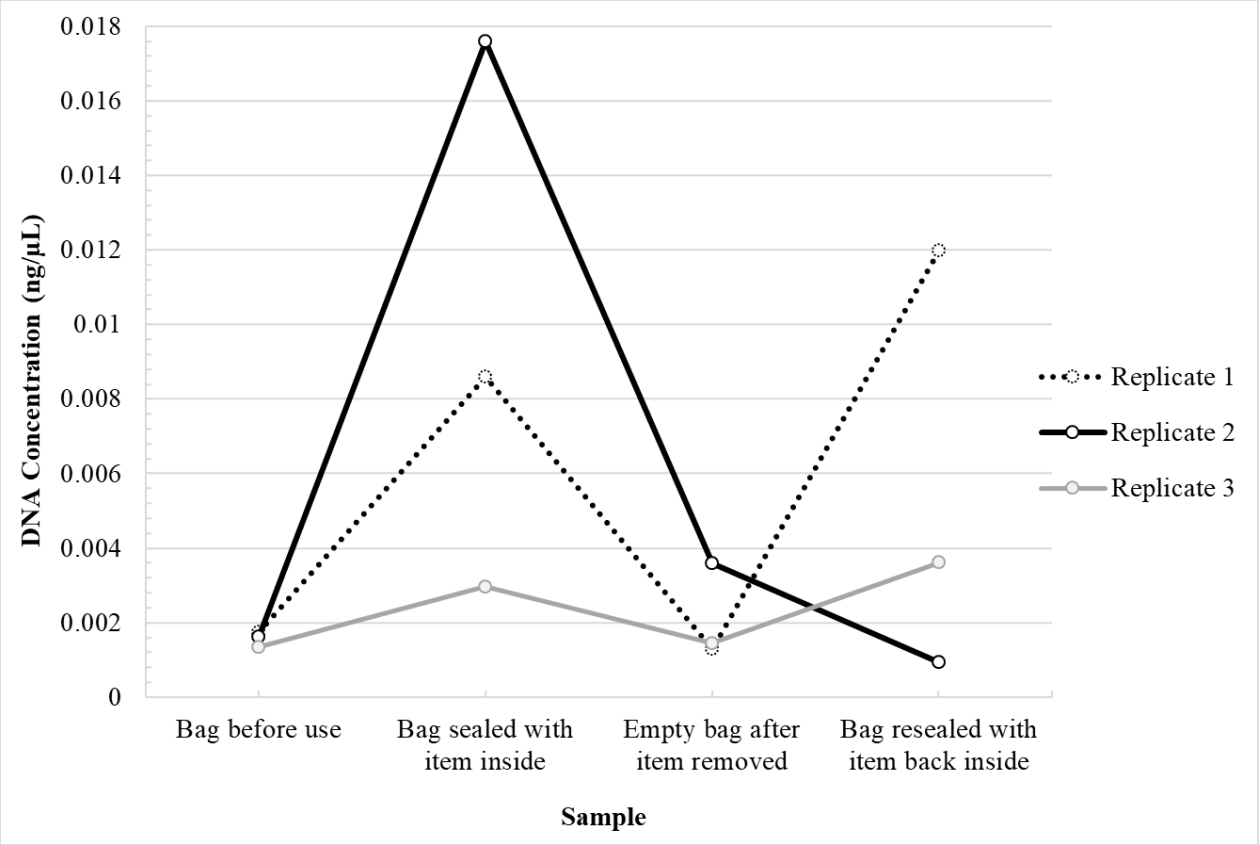

Figure 6.4. Concentration of DNA (ng/µL) recovered from the samples taken from the exhibit bag before use (1), after placing the shirt onto the outer surface of the bag, packaging it and sealing the bag (2), after removing the shirt from the bag (3) and after re-packaging the T-shirt and sealing the bag (4).

In the experiment where the t-shirt was place directly onto the bag, less than 0.0018 ng/µL of DNA was recovered from all of the exhibit bags before the T-shirt was placed onto the surface of the bag. In all replicates from this experiment, the concentration of DNA recovered from the sample taken from the exhibit bag after the item was packaged was higher than the concentration recovered from the bag before use. The concentration of DNA recovered from the bag after the T-shirt was removed was lower than the sample taken after the initial exhibit packaging, in all replicates. The concentration of DNA recovered from the samples taken after the T-shirt was placed back into the bag and the bag was resealed varied in each replicate. In replicates 1 and 3, the amount of DNA recovered from the last sample was higher than all samples previously taken within that replicate. In comparison, the concentration of DNA recovered from the sample in replicate 2 was lower than all other samples which were taken.

Table 6.2. The minimum number of contributors in profiles analysed using STRmix™ which were generated from samples taken from the exhibit bag before use (1), after placing the shirt onto the outer surface of the bag, packaging it and sealing the bag (2), after removing the shirt from the bag (3) and after re-packaging the T-shirt and sealing the bag (4). For profiles where the T-shirt donor was a DNA profile contributor, the LR for the inclusion has been shown.

Replicate	Sample	Minimum number of profile contributors*	Likelihood ratio for inclusion to T-shirt donor
1	1	4	
	2	3	4.32×10^{27}
	3	3	1.11×10^{9}
	4	(2)	8.03×10^{26}
2	1	3	
	2	4	3.74×10^{27}
	3	3	3.07×10^{24}
	4	3	3.08×10^{11}
3	1	4	
	2	3	1.58×10^{19}
	3	[3]	1.66×10^{9}
	4	2	3.43×10^{27}

*Contributor numbers displayed in brackets were from profiles which were reanalysed using a peak detection threshold other than 50 RFU. Square brackets [#] represent profiles analysed with a threshold of 20 RFU and round brackets (#) represent profiles analysed with a peak threshold of 175 RFU.

In all samples taken, a minimum of 2 contributors were observed within the profiles generated. DNA from the individual who donated the T-shirt used within these experiments was not detected on the bag before it was used. In all replicates, the individual who donated the T-shirt was identified as a profile contributor in DNA profiles generated from samples taken from the bags after the item was packaged, unpackaged and repackaged. As the concentration of DNA recovered from each sample increased, the strength of the likelihood ratio for the inclusion of the T-shirt donor also increased.

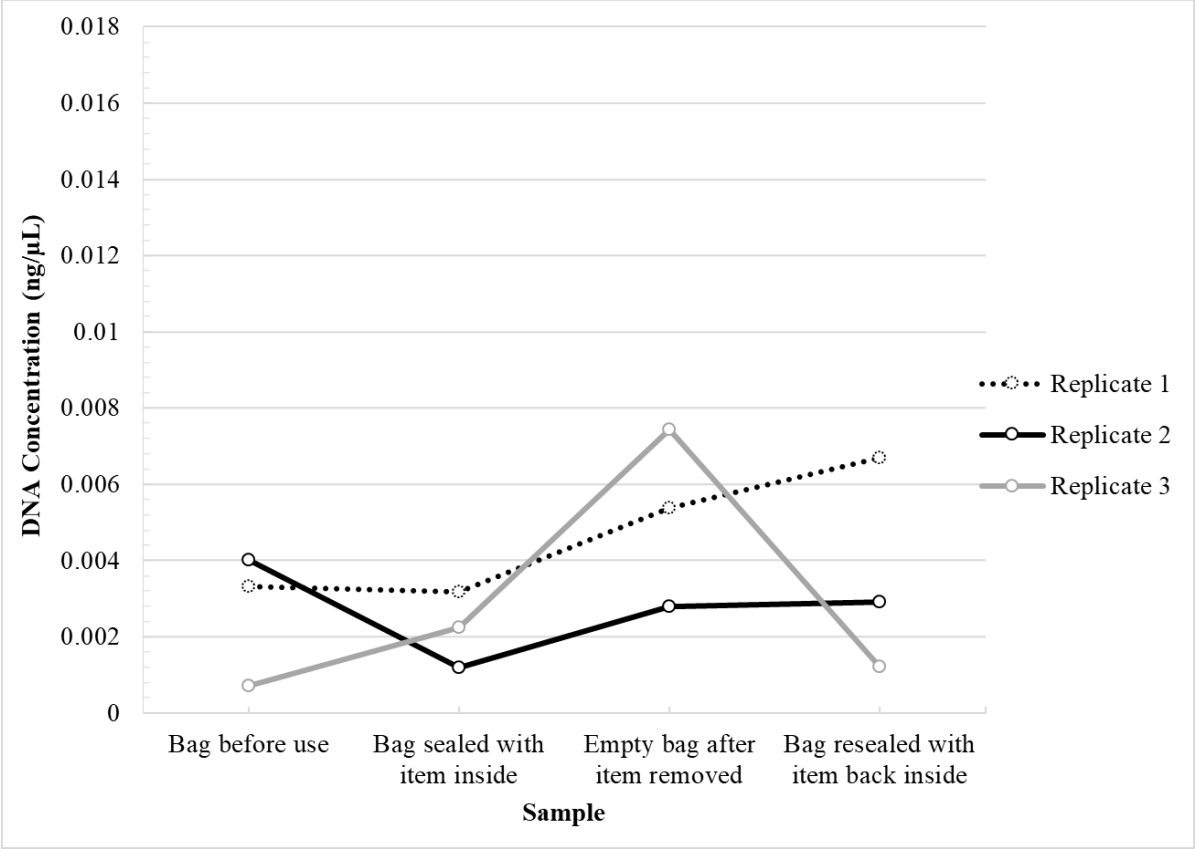

Figure 6.5. Concentration of DNA (ng/µL) recovered from the samples taken from the exhibit bag before use (1), after packaging the T-shirt and sealing the bag (2), after removing the shirt from the bag (3) and after re-packaging the T-shirt and sealing the bag (4).

The concentrations of DNA recovered in experiments where the T-shirt was not placed onto the outer surface of the bag before packaging (Figure 6.5) were lower than when the T-shirt was placed onto the bag (Figure 6.4). Between 0.00070- 0.0040 ng/µL of DNA was recovered from the samples taken from the bags before use. In replicates 1 and 2, the concentration of DNA recovered from the sample taken after the T-shirt was packaged was lower than the sample taken before the bag was used. In all replicates, the concentration of DNA recovered from the sample taken after the T-shirt was removed from the bag was higher than the sample previously taken. The concentration of DNA recovered from the sample taken after the T-shirt was repackaged was higher than the concentration recovered from the sample taken after the T-shirt was removed from

the bag in replicates 1 and 2. In replicate 3, the concentration of DNA recovered from the last sample was lower than all other samples within the replicate, except for the sample taken before the bag was used.

Table 6.3. The minimum number of contributors in profiles analysed using STRmix™ which were generated from samples taken from the exhibit bag before use (1), after packaging the T-shirt and sealing the bag (2), after removing the shirt from the bag (3) and after re-packaging the T-shirt and sealing the bag (4). For profiles where the T-shirt donor was a DNA profile contributor, the LR for the inclusion has been shown. Minimum number of profile contributors were assessed using a 20 RFU peak threshold.

Replicate	Sample	Minimum number of profile contributors	Likelihood ratio for inclusion to T-shirt donor
1	1	2	
	2	3	2.44×10^{18}
	3	2	1.89×10^{27}
	4	3	1.33×10^{24}
2	1	2	
	2	2	
	3	3	2.56×10^{11}
	4	3	9.03×10^{6}
3	1	3	
	2	2	8.78×10^{11}
	3	3	3.80×10^{25}
	4	2	3.34×10^{16}

In all profiles generated, a minimum number of 2-3 contributors were observed. DNA from the individual who donated the T-shirt used within these experiments was not detected on the bag before it was used. In replicates 1 and 3, the individual who donated the T-shirt was identified as a contributor to the profiles generated from the samples taken from the bag after packaging, removing and re-packaging the T-shirt. In replicate 2, DNA from the individual who previously wore the T-shirt was only detected within the samples taken after the T-shirt was removed from the bag, and after repackaging it and resealing the bag.

6.3.5 Discussion

The variation in DNA amount and profile complexity from samples taken from the exterior of casework exhibit bags is likely to be due to the particular handling history of each exhibit. Highly variable quantities of DNA were also recovered from samples taken from casework evidence bags in another study [12]. As PPE is not required during many stages of the handling of exhibit packages, there is the potential for DNA to be transferred to the bag directly through police officers, administration staff and evidence recovery staff, who handled the items prior to examination. It has been previously identified that DNA from individuals who have handled an exhibit bag without gloves can be detected on the exterior of the bag [12]. DNA could have also been transferred to the exterior of the bag through many intermediary surfaces, including storeroom shelves, workspaces within administration, other exhibit packages and through equipment used within areas exhibits are handled. While no previous studies have extensively examined the potential for touch DNA transfer between evidence bags and other substrates, transfer between many other substrate types has been observed to frequently occur [1, 9, 12, 14, 15]. During exhibit storage, which may involve long periods of contact and friction between exhibit packages, the contact types and conditions which have been previously shown to efficiently facilitate DNA transfer may be present [14, 16]. It has also been demonstrated that without regular cleaning, workspace benches, such as those where exhibits are stored, can accumulate high levels of DNA [17, 18]. As the exhibits are stored indoor, within a temperature-controlled environment, and are protected from UV light, these conditions may allow the DNA to persist on these surfaces for long periods of time [5].

It was expected that the majority of DNA within the sampling area would be removed as a result of tapelifting, therefore DNA recovered from samples taken after exhibit examination was either added to the package during the examination process, or not removed during the initial tapelifting. While not statistically significant, the increase in the concentration of DNA and profile contributors between samples taken before and after exhibit examination indicates that on most exhibit bags sampled, DNA was added to the exterior of the bag during the examination process. The increase in DNA on the exterior of the bag could have been the result of DNA transfer from the exhibit inside the bag during exhibit removal or re-packaging after examination. However, it cannot be excluded that the increase in concentration of DNA and profile contributors observed between samples could also be due to DNA, which was present on another part of the bag, and transferred into the

sampling area through handling the package during examination. The chosen sampling area may not have been an accurate representation of the DNA, which was present over all of the bags sampled, however, it still provides an insight into the minimum amounts of DNA accumulating on casework exhibit bags.

The evidence bags used in Parts 2 and 3 of the study were not considered to be 'DNA free,' therefore it was expected that DNA may be recovered from the samples taken from the bags before use. As there is more opportunity for DNA transfer events to occur over an increased time period, it was expected that the longer mock exhibits were stored within the exhibit storeroom, the more DNA would accumulate on the exterior of the bags. However, there was no statistically significant difference between the concentrations of DNA recovered from each time point. The variation in DNA concentrations could be due to differences in contact with other exhibit packages, individuals who frequent the storeroom and contact with areas of the shelf; all of which can result in DNA transfer both onto bags and from bags to other surfaces. The opportunity for individuals to deposit their DNA within workspaces they frequent has been identified in previous studies [17]. An increase in the number of profile contributors was observed between the baseline samples and the samples taken at both 4 and 8 weeks in each replicate, with the highest number of contributors observed after 8 weeks of storage. Our observation is that the longer exhibit packages remain in an exhibit storeroom, the more opportunity there is to collect low levels of DNA. The change in such low concentrations of DNA could explain the variation in amounts recovered from each sample point.

In Part 3, all LRs provided very strong support for the inclusion of the T-shirt donor as a profile contributor. The individual who donated the T-shirts used in the experiments was not a contributor to any of the DNA profiles generated from samples taken from the bags before use. This confirms that DNA from this individual was not present on the bags prior to packaging the T-shirt. The increase in the concentration of DNA recovered after placing the T-shirt onto the bag and packaging, indicates that during this process DNA was added to the bag. In all three replicates, the T-shirt donor was a contributor to the DNA profiles generated from these samples. This is most likely to be the result of DNA from the individual who wore the T-shirt, being transferred onto the bag during the direct contact before packaging. This increase in DNA was not observed on the samples taken from the bag in replicate 1 and 2 when this step was excluded, however, the T-shirt donor was still identified as a contributor within the profile generated from the DNA recovered in two of the three replicates. This indicates that even when the T-shirt was not placed directly onto the bag, transfer of DNA occurred during the packaging process. DNA from the T-shirt could have been transferred to the exterior of the bag through brief contact as the item was packaged or transferred onto the gloves worn at the time of packaging, and then onto the bag while sealing it. The transfer of DNA from an exhibit to another surface, via gloves, has been demonstrated in several previous studies [1, 7-11].

In both variations of these experiments, the T-shirt donor was a contributor to the DNA profiles generated from all samples taken after removing the T-shirt from the bag and re-packaging it. Tapelifting of the same bag during the packaging and examination process provides insight into changes in DNA amounts, but the mechanics of the tapelift also remove variable amounts of DNA at each step. This conflicting simultaneous addition and removal of DNA, which occurs between each of the sampling stages, makes it difficult to draw conclusions about the persistence of DNA on the bag. It was observed that the amount of DNA recovered at subsequent time points decreased in some experiments, which might be due to removal of DNA via the tapelift, and the exact amount of persistence of the donor's DNA across all sample time points may vary for the same reason.

Even when the T-shirt was not placed onto the bag during the process, there was an increase in the amount of DNA detected over subsequent time points, showing that DNA can be transferred to the exterior of the bag during the removal of the T-shirt. In both variations of these experiments, there was generally a higher concentration of DNA recovered from the sample taken after the T-shirt was re-packaged compared to after it was removed. This indicates that during the process of re-packaging an item after examination, DNA can be transferred from an exhibit to the outside of a package. This result reinforces the idea that DNA found on the casework exhibit bags after examination could have been transferred from the exhibit during the examination process. The results of Part 3 also indicate that the DNA recovered from the casework exhibit bags before examination, could have been transferred from the exhibit to the exterior of the bag during packaging at the crime scene.

This study provides an insight into the DNA load accumulating on evidence bags throughout the handling and storage of exhibits. Previous research identified the importance of investigating the accumulation of DNA on evidence bags, during criminal investigations [12]. It has been demonstrated that even when strict contamination minimisation procedures are followed, DNA can be transferred from the exterior of a bag to an exhibit during the examination process [12]. The chance of these incidental contamination events was observed to increase with the amount of DNA found on the outside of the bag [12]. In this current study, it was observed that detectable concentrations of DNA can accumulate on the exterior of evidence bags during the packaging, handling, storage and examination of exhibits. Current literature demonstrates that DNA can be transferred between several different substrate types during handling [1, 9, 12, 14, 15], thus raising questions around the potential for DNA to be transferred from the exterior of evidence bags to forensic workspaces. As DNA has also been shown to persist on items for long periods of time[5], with the results from this study indicating that DNA from an exhibit can be transferred to the exterior of the evidence bag through packaging, it further raises the question about whether DNA from exhibits themselves is accumulating in forensic workspaces. As contaminations can have serious consequences on criminal investigations, many studies have examined the opportunity for

exhibit and sample contaminations within forensic laboratories and at crime scenes [12, 19-21]. Much of this research is responsible for the improvement of Quality Assurance programs and contamination minimisation procedures, which are utilised in forensic laboratories worldwide. The importance of contamination best practice in handling of exhibit bags is as critical as any other step in the evidence handling pathway. Studies which provide an insight into the transfer of DNA to and from evidence bags enable best-practice procedures for exhibit collection, transport, storage and handling to be established.

To better understand the potential for incidental contaminations through evidence bags, further research is required. This includes investigating the potential for DNA to be transferred from evidence bags to other surfaces and the ability for DNA from an exhibit to be transferred through the surface of an evidence bag and deposit on the exterior bag surface.
This study shows the importance of all individuals who handle exhibits being on an elimination database to enable the detection of incidental contamination events which may occur as a result of DNA being transferred from an evidence bag to an exhibit.

6.3.6 Conclusion

During exhibit transport, handling, storage and examination in criminal investigations, significant amounts of DNA are added to the exterior of evidence bags. This reinforces the risk of DNA being inadvertently transferred from an exhibit package to the exhibit, causing an incidental contamination. To minimise the risk of these contaminations, further research involving DNA transfer between evidence bags needs to be conducted. This is required to ensure that current evidence handling procedures are still effective with the highly sensitive DNA profiling systems now being utilised.

6.3.7 Acknowledgements

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6.3.8 Conflict of Interest

The authors declare none.

6.3.9 References

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6.3.10 Poster presentation

The following poster was produced from a portion of the data from the previous publication, and was presented at "The ***28th Congress of the International Society for Forensic Genetics***, Prague, Czech Republic, 2019. *DNA Transfer Between Evidence Bags During Casework.*

DNA Transfer Between Evidence Bags During Casework

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Presented at the 28th Congress of the International Society for Forensic Genetics 2019 conference, Prague, Czech Republic. ABSTRACT NUMBER: P621

ABSTRACT Inadvertent transfer of DNA from the exterior of an evidence bag to other items provides the opportunity for exhibit contamination. It is assumed that evidence bags are DNA transfer vectors, but research which details the extent of this transfer is limited. With increased DNA profiling sensitivity, there is the increased chance of detecting DNA from a contamination within a casework sample.

To provide insight into levels of DNA which accumulate on evidence bags through handling and storage during casework, the exterior of evidence bags were sampled. Casework exhibit packages were sampled before and after the examination of the exhibit inside. Mock evidence bags were sampled after 1, 4 and 8 weeks of storage and throughout a process which replicates realistic exhibit handling.

DNA quantities recovered from casework exhibit packages were highly variable and produced highly complex profiles, containing many contributors. Although not statistically significant, DNA quantities and minimum profile contributors were higher from samples taken after exhibit examination, indicating that during examination DNA is added to the surface of the bag. It was observed that through the process of packaging and un-packing an exhibit, DNA from the item was transferred to the bag exterior. A trend observed was that the complexity of DNA profiles produced from mock exhibits increased with time of storage.

Studies examining the levels of DNA accumulating on evidence bags enables best practice to be implemented during the handling, transport and storage of casework exhibits.

Introduction

The increasing sensitivity of DNA profiling technology increases the chance of detecting DNA from a contamination event. Many studies demonstrate the ability for DNA to be transferred between substrates directly and in-directly through one or more DNA transfer vectors [1-3] . In a number of studies gloves and examination tools have been identified to act as efficient DNA transfer vectors [4,5]. In comparison, there are limited studies that investigate the ability for evidence bags to act as transfer vectors. A recent study demonstrated that during the examination process, even while strict contamination minimisation procedures are followed, DNA can be transferred from the exterior of an evidence bag to the exhibit inside [6]. In this study we determine the levels of DNA accumulating on the exterior of exhibit bags during casework.

Materials and Methods

Sampling Process:

- DNA Tapelifts (Lovell Surgical Solutions) were used to sample the area shaded in red on all evidence bags.

1. Casework evidence bag study:

- Samples taken from 10 randomly chosen casework evidence bags before and after exhibit examination.

2. Exhibit storeroom study:

- Nine mock exhibits produced by packing a lab coat into a paper evidence bag.
- A sample was taken from each bag
- Bags placed into FSSA evidence storeroom, with three exhibits on each of three shelves.
- After 1 week, an exhibit bag from each shelf was sampled.
- After 4 weeks another bag from each shelf was sampled.
- The remaining three bags were sampled after 8 weeks.

3. Transfer during exhibit packaging and examination:

- Cotton T-shirts worn while running used as mock exhibits.
- In three of the six replicates performed, the T-shirt was placed onto the bag for 30 seconds before packaging to replicate an exhibit being placed onto the bag for photographing.

Sample Analysis:

- Performed using in-house validated procedures at FSSA.
- DNA extracted using the DNA IQ system (Promega) on a Multiprobe II 8 tip Liquid Handling Platform (LHP) (PerkinElmer).
- A Janus® 4 tip LHP (PerkinElmer) used to prepare samples for quantification, using Quantifiler™ Trio (Applied Biosystems™) on an ABI PRISM® 7500 Sequence Detection System (Applied Biosystems™).
- DNA amplified on a ProFlex™ Dual 96-Well PCR System (Thermo Fisher Scientific) using GlobalFiler™ (Applied Biosystems™).
- DNA fragments resolved on an ABI 3500xl Genetic Analyser (Applied Biosystems™) and analysed using GeneMapper® ID-X Software
- Peak threshold of 50 RFU used in Parts 1 and 3 and 20 RFU in Part 2.
- Profiles in Part 3 analysed using STRmix™, those containing >4 contributors reanalysed in GeneMapper® ID-X using a peak threshold of 175 RFU prior to analysis in STRmix™.
- Profiles were compared to a reference profile from the T-shirt donor and a Likelihood Ratio (LR) for the match was generated.

Results and Discussion

1 Casework evidence bag study

- Compared to samples taken from the bag before exhibit examination, a higher quantity of DNA and number of profile contributors were observed on most samples taken after examination.

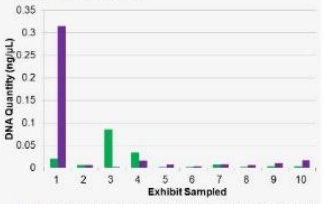

Fig. 1 DNA recovered from samples taken from 10 casework exhibits before and after exhibit examination.

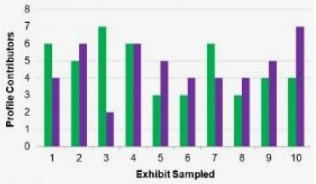

Fig. 2 Minimum number of contributors observed in profiles generated from casework exhibits before and after examination. Profiles analysed using a threshold of 50 RFU.

2. Exhibit storeroom study

- DNA was added to most exhibit bags during storage.
- The complexity of DNA profiles increased as the time of storage also increased.

Table. 1 DNA recovered from samples taken at baseline (B) and after 1, 4 and 8 weeks in each of the three replicates (R1, R2, R3).

DNA Quantity (ng/µL)	B	1 week	B	4 week	B	8 week
R1	0.0040	0.0022	0	0.0005	0	0.0042
R2	0.0006	0.0002	0	0.0024	0	0.0013
R3	0	0	0.0023	0.0013	0.0003	0.0044

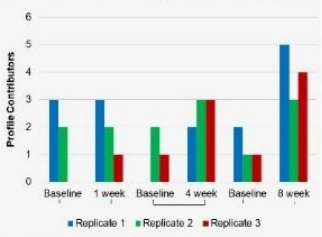

Fig. 3 Minimum number of contributors observed in profiles generated from the samples taken at each time point in each replicate.

3. Transfer during exhibit packaging and examination

- DNA was added to the bag during the exhibit packaging and un-packing stages.
- DNA from the T-shirt donor was detected on all samples, except those taken before the bag was used.

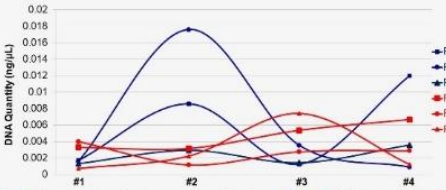

Fig. 4 DNA recovered from the bags at each sampling stage (#1, #2, #3, #4). In 3 replicates the T-shirt was placed directly onto the bag before packaging (R1, R2, R3) and the other 3 replicates it was not (R1, R2, R3).

Table 2. Likelihood Ratio (LR) generated for the match to the T-shirt donor at each sampling stage.

Replicate	Sample	LR for match to T-shirt donor (shirt placed directly onto bag)	LR for match to T-shirt donor (shirt not placed onto bag)
1	1	-	-
	2	4.32×10^{27}	2.44×10^{19}
	3	1.11×10^{6}	1.89×10^{27}
	4	8.03×10^{28}	1.33×10^{24}
2	1	-	-
	2	3.74×10^{27}	-
	3	3.07×10^{24}	2.56×10^{11}
	4	3.08×10^{11}	9.03×10^{5}
3	1	-	-
	2	1.58×10^{19}	8.78×10^{11}
	3	1.66×10^{5}	3.80×10^{23}
	4	3.43×10^{27}	3.34×10^{18}

Concluding Remarks

- Variable levels of DNA are added to the exterior of evidence bags throughout the routine handling and storage of exhibits during casework.
- DNA from the exhibit was detected on the exterior of the evidence bag after the initial packaging of the item and the after the exhibit packing and un-packing process.
- Further research is required to better understand and minimise the risk of contaminations caused by evidence bags.

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6.4 Certificates

6.4.1 ISFG Travel Bursary

INTERNATIONAL SOCIETY FOR FORENSIC GENETICS

ISFG

Claire Mercer

has been awarded a Travel Bursary to attend the 29th Congress of the International Society for Forensic Genetics in Washington, DC, United States of America

The President
(John M. Butler)

WASHINGTON, DC
ISFG 2022
INTERNATIONAL SOCIETY FOR FORENSIC GENETICS

The Secretary
(Peter M. Schneider)

Washington DC, 1st September 2022