Sampling techniques for the recovery of male offender DNA from the female victim skin surfaces

By

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Declaration

I declare that this thesis does not contain any material submitted previously for the award of any other degree or diploma at any university or other tertiary institution. Furthermore, to the best of my knowledge, it does not contain any material previously published or written by another individual, except where due reference has been made in the text. Finally, I declare that all reported experimentations performed in this research were carried out by myself, except that any contribution by others, with whom I have worked is explicitly acknowledged.

Signed: NeilJames Galgey

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Literature Review

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Masters in Forensic Science Literature Review

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Abstract

Sexual assault cases can cause the victims to develop a myriad of different health and welfare issues and contributes to one of the reasons behind why sexual assault is considered a serious and inhuman crime. While the sexual assault is occurring it is possible that small minute pieces of the offenders' cellular material is transferred to the victim, their belongings or the crime scene where it happened. This transfer of DNA is often caused by skin-to-skin contact or by oral acts allowing for saliva to be transferred to the victim. Therefore, the victim's skin is a critical crime scene for obtaining the offenders DNA to produce a usable DNA profile and thus it is imperative for the sampling technique performed on the victim's skin to be performed both correctly and minimal contamination and loss of cellular material occurs. This literature review aims to determine what research surrounding the different sampling techniques are available and whether there are any factors or pitfalls that impact the prevalence of obtaining cellular material.

There are multiple different factors that influence how well the sampling and extraction methods go such as background DNA, shedder status and washing. Contrary to the contradictory results of the studies in this area it is usually agreed that for a sampling technique to be at its most effective, a ratio of increased offender DNA to decreased victim DNA as possible is desired. From this it can be argued that swabbing is more effective than tape lifting however the research where this was all supported by evidence of previous studies providing a clear result of which sampling method is the most effective does not exist. Some studies exist where the single swab is compared to the double swab and tape lifting is compared to both stubbing and mini tape lifting however there is no study apart from the Harris study where most of the sampling techniques are looked at and compared to each other as a whole. This study is aiming to provide some possible clarity on which sampling technique is the most effective however there has to be more research in this field to both validate and allow for these results to be reliable also.

1.0 Introduction

Sexual assault is a severe public health problem affecting millions of people worldwide, particularly women, and is regarded as one of the most heinous type of crime due to the amount of extreme psychological, physical and emotional trauma that often impacts the victim long after it occurs.[1],[2] The criminals who carry out these crimes are usually ostracized and ill-regarded even among the prisoners convicted of violent criminal offences.[2] After conviction the offender is labelled as a sex offender and this is associated with shame, stigma and public rejection.[2] Due to a range of intrinsic circumstances and common issues such as the investigators undergoing 'tunnel vision' and mistaken witness identification especially in stranger rape as a consequence of the lack of physical evidence and a multitude of possible suspects.[2] This is why DNA evidence is so important in these types of cases[1],.[3]

In Australia, it is estimated that 17% of all women aged over 18 and 4% of all men aged over 18 have experienced sexual assault since the age of 15.[2, 4] It has been found that women would be most frequently sexually assaulted by friends or acquaintances of the victim followed by intimate partners and then strangers, respectively.[3] Sexual assault is a widespread, underreported and under prosecuted crime.[4] The population-based surveys on self-reported rapes has found that only one in ten rapes is reported to police or

healthcare and among these rape victims reporting to the police, 50-70% had also been present at a sexual assault centre after rape and vice-versa.[4] However, despite the numbers of sexual assault cases reported to police increasing, the number of cases proceeding to court is quite low and nearly constant causing a decreasing proportion taken to court.[4] Following attending a sexual assault centre a forensic medical examination is carried out which is for both the victim's health and legal interest as it can provide critical evidence for the investigation and prosecution of a sexual assault case. [4] However, from the forensic intervention angle while there are some published protocols and guidelines only a couple countries have officially adopted specific guidelines regarding evidence management. [3] Once these official guidelines have been adopted, they can vary in a multitude of different ways in that same country, between the regions of the country and between different institutions. [3] Some of the most important guidelines provided by the Magalhães et al.[3] study is that evidence preservation by the victim is paramount as the DNA evidence on the victim is very important and any loss, contamination or destruction of this would severely impact the probative value at trial. To avoid destruction of evidence, the professional who the victim reported the sexual assault to would need to inform the victim or the person who reported the crime to avoid doing certain practises such as showering or bathing.[3] Steps to minimise the risk of DNA evidence contamination are to always use disposable supplies, work under aseptic conditions, ensure the room where the samples being taken on the victim is regularly cleaned, avoid sneezing, talking, coughing and eating near the samples and store each sample separate from each other to avoid the samples contaminating each other.[3]

The World Health Organisation defines sexual violence as any sexual act, attempt to obtain a sexual act, unwanted sexual comments or advances, or acts to traffic, or otherwise directed against a person's sexuality, using coercion, by any person, regardless of their relationship to the victim, in any setting, including but not limited to home and work.[5] There are many subcategories of sexual violence such as sexual assault which is defined by the World Health Organization as sexual assault usually includes the use of physical or other force to obtain or attempt sexual penetration.[5] It includes rape, defined as the physically forced or otherwise coerced penetration of the vulva or anus with a penis, other body part, or object, although the legal definition of rape may vary and, in some cases, may also include oral penetration.[5]

One thing that is usually found providing the victim of sexual assault did not instinctively shower or bathe post sexual assault is DNA evidence of the perpetrator as it is possible that a lot of the forensic biological evidence may get washed off the skin.[3],[6] As sexual assault victims can come into a Sexual Assault Resource Centre (SARC) after washing themselves according to both which means that there will be less offender DNA present on the victim or their clothes.[3][7] It has been shown through various studies that DNA can survive on the skin surface for a good 72 hours[7] however getting a full forensic medical examination after sexual assault for best results, less than 24 hours is usually advised. [3-4][7]

One of the most helpful uses of DNA evidence is the presence of semen left behind at the scene of the crime, on the victim's body, clothes or vicinity during a forensic medical examination, in a court of law confirms the diagnosis of sexual contact occurring. [3] This type of DNA evidence is also especially helpful in suspect identification however this does not mean that this provides irrefutable proof that a sexual assault has occurred especially in

incest or interfamily case types as it has been previously shown in Kafarowski et al. [8] and Brayley-Morris et al.[9] that it is possible for secondary transfer of spermatozoa from adult clothing or bedsheets to babies, children, siblings clothes and/or bedsheets during machine washing essentially if the offender was anyone living with the victim.[3]

Semen is defined biologically as spermatozoa suspended in seminal fluid and it is found to rarely persists in the sexual assault victim's oral, anorectal, and vaginal cavities 6,24,72 hours post sexual contact. [3[8, 10-12] Semen happens to have a different half-life time depending on what cavity it is found in, with the vaginal cavity also depending on the age of the victim as the semen persists for less time in pre-pubertal girls due to them lacking cervical mucus due to lack of puberty. [3, 8, 10-12] It also depends on if the semen gets localised in the cervix, it can potentially survive more than 72 hours and in post-pubertal females; spermatozoa can remain motile in vaginal secretions for 6-12 hours and in the cervix for up to 5 days; non-motile spermatozoa can be found in the stains of vaginal secretions of post-pubertal females for 12-48 hours' post-ejaculation. [3, 8, 10-12]

The stains of dried secretions on clothing been shown to be quite stable and thus it is possible to detect semen from the clothing for up to 1 year. [3, 8, 10-12] However, the half-life estimations mentioned here is from a lab not from real cases where a variety of factors that are usually described in the medical report must be considered when determining whether the presence of semen is present in a sexual assault case. [3, 8, 10-12] Some of these factors that impact the presence of semen being present after a sexual assault are the type of act occurring and the circumstances leading towards it (such as where the evidence is deposited, the ejaculation occurs in the skin, oral, anal, or vaginal mucosa or in the cervix, also whether a condom was utilised, the perpetrator has been vasectomised or they are azoospermic, the time between the sexual assault and evidence collection, the victim's gender, age and activities such as excreting, vomiting, bathing, brushing teeth especially if oral assault occurred, eating, drinking, smoking and exercising after the sexual assault occurred.[3, 8, 10-12]

Semen and spermatozoa are not the only type of forensic evidence left behind by the perpetrator at the scene of the crime or secondary crime scenes as in all crimes against people such as sexual assault, the contact between the victim and the offender or his/her environment or both always leaves something behind, as either evidence will be transferred from the offender to the victim or the scene or vice-versa. [3-4] This evidence that is transferred by the offender to either the victim[13] or the environment is typically called trace evidence, and this is essentially anything be it an item or information that helps the investigation to find the truth of the matter. [3] as this evidence is helpful in directing the police investigation, provide a reliable identification of the offender, exonerate any suspect or accused that is innocent of the crime, support or contradicts any of the statements made by the victim, witnesses or the suspect and subsequently allows the police to determine whether the lead is a dead end or not and thus guide the future of the investigation, provide information about the crime scene and provide the proof that the alleged event actually occurred. [3]

Usually, crime scene evidence can be found anywhere a criminal offence has occurred, on anything that the victim was wearing or carrying during the time when the offence occurred or within or on the body of anyone that is connected to the offence. [3] This evidence can be found on the victim's body and/or clothes, in condoms or bedclothes or at the crime scene

therefore the examiner must inform the investigating officers to isolate and protect the crime scene and should collect the more urgent samples first as some biological evidence deteriorates more rapidly than others. [3] The examiner must also consider that in sexual assault cases the victim's body is potentially the most important crime scene. [3]
This indirect evidence is usually the most abundantly analysed in forensic laboratories and can be further subdivided into two different categories. [3] Physical evidence is items of non-biological origin such as finger and footprints, shoe/tire impressions, fibres, paint, soil, dirt, glass, headlamps or arson debris, explosives and gunshot residue. [3] This type of evidence is helpful in both identifying the crime scene and should be collected when they are present. [3] Another subtype is biological evidence is usually present in sexual assault cases and is quite important in regards to sexual assault cases, biological evidence is essentially anything that is of biological origin and is usually found to be from either the victim or the perpetrator such as semen, vaginal fluid, oral fluid, sweat, blood and other body fluids, hair, cells of the alleged offender under the victim's fingernails (especially if the victim scratched the offender in the ensuing physical alteration prior to the sexual contact) or epithelial cells from the victim being present on the offenders penis and botanical elements like pollen, plants or wood.[3] What must also be remembered is that females are not the only possible victims of sexual assault, it is possible for males to be sexually assaulted however it is far more likely for females to be the victim. [1]
Biological evidence taken from the victim's body or clothes is so important in sexual assault cases due to the fact that it allows for the police investigators to place the suspect at the scene if their DNA (biological evidence) is present at the scene, identify the perpetrator, eliminate an innocent suspect allowing for different case avenues to be explored, assist in the prosecution of the sexual assault case and/or corroborate support evidence which helps support the question of fact, that physical contact between the victim and the accused has occurred. [1, 7, 14] As noted previously semen is not the only type of biological evidence left behind at the scene of the crime or surrounding the victim's body or their clothes or belongings and thus the forensic examiner would need to take some considerations into account in order to get the most amount of information from the observed evidence. [3]
The prostate-specific antigen is a serine protease produced by the prostatic epithelial cells found in many tissues such as seminal fluid, prostatic fluid, male serum, male urine, apocrine sweat glands and breast milk from lactating women. [3] As prostate-specific antigen is not specific to either tissue or gender, in sexual assault cases, the interpretation of the results of this test should not a significant problem because of the low levels of the enzyme in non-prostatic fluids. [3] This enzyme can be found in post pubertal females' vagina or cervix for up to 48 hours post sexual contact. [3] This technique is considered among the most sensitive methods for semen detection and can be applied to azoospermic individuals also. [3] This method should only be taken as a guide as if the result is negative then DNA tests such as Y-STRs or autosomal STRs should be performed just in case. [3]
In sexual assault it is possible that the offender's saliva may be transferred to the victim's skin by licking, kissing, sucking, biting or spitting, of these only biting or sucking are likely to leave behind any possible marks on the victim's body. [3], [7] When no marks are easily visible on the victim's body, it all comes down to the victim's statement about what the offender's actions during the assault were in order to guide the forensic medical examiner to determine fundamentally the likely area for where to sample/swab on the victim to get the most

amount of trace evidence. [3], [7] This oral fluid is the second most common biological evidence found in sexual assault cases. [3]

A common test for the presence of saliva is the Phadebas test which determines alpha-amylase test but it should also be considered that alpha-amylase can be found in other bodily fluids.[3] This body fluid is quite helpful when found on or near the victim as it transports epithelial cells from buccal mucosa from the offender and this contains their DNA which is helpful in identifying the assailant.[3, 15-20] There are arguments in the literature that the assailant's DNA may be observed in the victim's oral cavity for up to an hour after intense kissing.[3(15-20)] However, it is very difficult to observe in practise as the victim usually presents for a full forensic medical examination quite some time after the sexual assault and after they have washed their mouth. Therefore, the collection of this oral fluid must be collected as soon as hygienically possible for the victim's comfort and in order to avoid the destruction and loss of this important biological evidence. [3(15-20)] Some possible pieces of evidence that can be of potential use for this type of evidence are cigarette filters, bottles, cans, essentially anything the offender has licked or potentially licked or used their mouth on and is left at the scene of the crime. [3, 15-20]

Trace or touch DNA refers to the DNA from epithelial (skin) cells that are left behind after a person touches or comes into contact with items such as clothes or weapons essentially any item that can be touched or contact with the person occurs and usually force is present and thus usually trace DNA is present either at the crime scene or on the victim's clothes after a sexual assault.[3],[7],[21] There are a range of different factors that impact the success rate of obtaining a DNA profile from the trace evidence obtained in the case proceedings. [13] Such as the environmental conditions as in rainy wet conditions far less epithelial cells would be available to sample unlike if the same assault happened during dry conditions as then even the handling time could be negated as the time taken for the shredded epithelial cells to the touched item is instantaneous. [13] As consists of both being the largest organ of the human body in addition to consisting of around 15% of human body weight and thus depending on whether the assailant is a good or bad shedder up to 400,000 skin cells are shed daily, also those with recently washed hands provide very little DNA and it is difficult to detect where the trace DNA evidence is present on clothing as it is so small and thus why victim or witness statements are so important for this reason. [3], [13]

Hence why the collection and recovery of the DNA trace evidence is so important and so integral to which sampling method utilised as if pick the wrong sampling method and thus no trace DNA was taken then no evidence available for the identification of unknown offender or at least makes it more difficult.[13] Due to this it is very vital to collect the DNA trace evidence from the victim's body, with it being only the offenders' DNA, and why reference samples from the victim's prior sexual partner/s is so important as reference samples and therefore any trace DNA evidence and/or reference samples must be sampled as to be vitally secured as aseptically as possible to prevent contamination of the sample. [3, 7, 21, 22]

Primarily obtaining a sample of the offender's trace DNA from any trace evidence left at the crime scene or on or near the victim's body is very important and is very critical of what the sampling method used on the victim's skin to gather this evidence type.[14] Currently there are a lot of different sampling methodologies in use for collecting offender DNA from the victim's skin after the physical contact that has taken place during a sexual assault such as

various different methods involving swabbing and tape lifting. [14] The comparison of efficiency between these five different methods has been investigated before by Harris (23) and this study is going more in depth by increasing the sample size and determining what sampling method between swabbing, double swabbing, tape lifting, mini-tape lifting and alcohol wipes is both the most efficient and effective sampling technique for sampling offender DNA from a victim's skin. The common method in use by forensic examiners is the double swabbing technique. [22] Another possible method that can be mentioned is a vacuum by M-Vac© Systems Inc., (MSI), made with the purpose of obtaining the microbes present on fruits and vegetables can potentially be used for forensics use. [6] However as this study is undertaking a similar methodology as the Harris (15) study only the previous five methods will be used. In contradictorily to recent studies, double swabbing has been illustrated to recover far more DNA trace evidence when compared to the single swabbing method which means that the double swabbing technique increases the likelihood of a conclusive full DNA profile being found utilising the trace DNA evidence. [24]

2.0 Discussion

2.1 Recovery of DNA from skin-to-skin contact

DNA profiles are the reason why this trace biological evidence is so important as it provides a full representation of the presumed offender's DNA sequence if enough DNA(cells) is present, this is usually represented in a graph known as an electropherogram. [3],[25] When more than one individual contributes to a profile it is known as a mixed DNA profile and as during sexual assault it is likely to have both the victim and offender DNA as trace evidence, mixed profiles are quite common. [3, 7, 21, 22, 25] Mixed DNA profiles have been known to be quite difficult to individualise which individual provided which part of the profile as different people may and any known sexual partners are so helpful as negatives. [3,17] Thanks to the technology related to obtaining a DNA profile becoming more and more sensitive over the years, less and less DNA is required to obtain a full DNA profile and thus more degraded DNA can provide more information as the years go on. [26] As of the current literature around how many cells are needed for a full DNA profile of the assailant to be produced, it has been determined through the use of multiple studies that the agreed upon number is around 200 buccal cells or 150-200 sperm cells which is 0.5-1 nanogram of cellular material. [19, 27, 28] According to the most recent study it was found by Kanokwongnuwut et al. (21) that it was possible to obtain this and thus not many follow-up articles have been possible and hence it may be possible and more specific evidence would be available in the future.

As DNA is a material born from cells it can inferred that it can be transferred from one surface to another hence trace DNA evidence. [3],[29] Hence why so much probative value is put on whether any DNA trace evidence was present however there is intense scrutiny around how the DNA evidence was found on that object or the victim as while if the person's DNA is on the object it does not mean that no one else has touched that object in the meantime. [30] For example, in a case study a knife is found at the scene of a murder and it has been found that a considerable amount of people have previously utilised the knife prior to the stabbing and from the Buckingham et al. (23) study it was found that the later handlers of the knife provide more prominent DNA profiles than the ones who have handled the knife earlier, however the final handler of the knife is not necessarily the major contributor.

A direct or primary transfer of this DNA cellular material occurs before, during or after a crime has occurred via physical contact generally. [23] Indirect or secondary transfer of the cellular material through an intermediary handler which collects this transferred cellular material from the primary surface and transfers it to a secondary surface. [23] Some factors which affect the process of this primary and secondary transfer process is the composition of the two potential surfaces, the manner of transfer such as how much force was applied between surfaces, the type of biological material and its moisture level in addition to a person's shedder status. [23-(31)] This usually occurs in everyday life as people experience their normal everyday interactions and activities such as a person's hands regularly collects non-self cellular material from say a door handle and simultaneously placing both self and non-self cellular material on a secondary surface. [23] Consequentially, this DNA transfer of cellular material can happen a multitude of times especially in areas of increased turnovers rates such as public transport or doorknobs and thus this impacts how easily a conclusive DNA profile can be obtained. [8,23] Therefore for this reason, in order to obtain a successful DNA profile that provides critical information it still comes down to whether or not the target DNA of the offender is still present and secondary to that is the presence of background DNA which is essentially the victim's DNA or secondary transfer DNA. [27]

2.1.1 Background DNA

DNA profiles can be easily produced as long as there is an abundance of DNA from trace biological evidence, however in order to obtain a clearly conclusive DNA profile, normally one source of DNA is needed to be present in greater quantities. [3] In crimes such as homicide where large disposals of blood and/or semen is left behind at a crime scene, it makes answering the questions related to multiple DNA transfer somewhat straight forward in this circumstance. [32] However thanks to technology advances the analytical techniques relating to trace DNA evidence are becoming much more sensitive and discriminating and can detect full DNA profiles from increasingly smaller and smaller amounts of trace DNA evidence. [25] Therefore trace DNA evidence is becoming far more useful in criminal cases and thus trace DNA is becoming increasingly responsible for the sources of the majority of DNA profile production. [25] Primarily from the epithelial skin cells left behind by a victim/offender/witness via touching a surface however the most dominant profile is not always the last person to touch the surface. [23,25]

Electropherogram peaks from the victim in addition to the potential contamination of DNA, present on the skin of either the victim or the offender, may be present in the electropherogram of the DNA profile. [22] Due to the supposition that self-DNA transfers at a similar rate to non-self DNA that is already present on a surface it is usually referred to as background DNA. [7, 33] As a consequence of this it is sometimes highly difficult to distinguish to a reasonable degree of certainty between the direct offender DNA and the background DNA already present on that surface. [7, 33] From this it can be inferred that sometimes from the trace DNA evidence found at the crime scene or the victim's body, more than just the offender's and victim's DNA can be potentially found and thus the determination that the higher the offender DNA to victim DNA ratio, the decreased levels of complexity from the interpretation of the DNA profiles. [22]

In addition to this there are other potential factors behind the impact of background DNA found at the scene or victim's body such as lifestyle habits, living circumstances and the individuals' shedder status also impacts whether an individual's DNA would be present on

their skin cells to cause the background DNA to be present. [33] A study conducted by Graham and Rutty[26] reported that individuals who live with either their partners or family had increased levels of background DNA and this is a problem especially in sexual assault cases since the majority of the offenders are usually known to the victim by being friends, acquaintances or family. [3,17] A study conducted by Zilkens et al. (34) using a sample size of 1163 women determined that 32.3% of the time the offender was a friend or acquaintance of the victim, 17.5% of the time the offender was an intimate partner, 16.8% of the time the offender was a stranger, 18.9% of the time the offender was an accidental acquaintance (someone the victim has known for less than 24 hours), 8.6% of the time the victim had no memory of the offender and 5.8% of the time the offender was another in that they did not fit into any of the other categories.

In sexual assault cases, transfer of DNA occurs frequently via strong skin-to-skin contact or through other oral acts resulting in saliva being transferred or through sexual contact and sperm/semen being transferred. [3,7] From this it can be extrapolated that it is critical for the forensic examiner to know and understand the impact background DNA has on DNA profiling interpretation when handling trace DNA from skin cells. [33] Thus when analysing a mixed electropherogram it is critically important that every allele present and observed is determined impartially to be present, inspected and accounted for. [26] As background DNA cannot be fully avoided it is possible to minimise the risk of an abundance of it being present via the utilisation of the amount present by the chosen sampling technique.

2.2 Recovery of DNA from Saliva

During criminal acts where a crime is being committed such as sexual assault, physical skin-to-skin contact is not the sole contributor of forensic trace DNA evidence on the victim's body or crime scene. [3,7,13] The offenders' saliva may be present on a victim's skin due to the transfer of saliva during the offender's actions while the sexual assault was taking place such as by licking, kissing, sucking, biting or spitting. [3, 6, 14, 17] A potential problem regarding identifying where on the victim's body the saliva is present is difficult as it is something not easily seen with the naked eye and thus the forensic examiner is relying on victim and witness statements about where the likely location to sample is. [2] As it is possible for them to be mistaken and not remember clearly what was happening during the result it is quite possible that the wrong location is sampled for saliva and thus that vital evidence is gone and lost. [1,2,6]

A potential solution to this problem is through the use of a dye for the use of targeted sampling however it is not practical for large objects as a result of this the development of an efficient device for the efficient use of a dye distribution system is required for its utilization in practise. [20] The study by Young and Linacre[37] found that the feasibility of using a device to cover relatively large objects with the Diamond Nucleic Acid Dye (DD) exists and therefore increases the likelihood of observing cellular material at both the micro and macro level. In addition to this the study[37] also determined that to observe the most desirable results the device would need to have a pressurized continuous-spray mechanism in order to obtain a considerably constant size of the dye droplets which influences the intensity and surface coverage of the dye. This dye has not been used on skin but considering it works well on other substrates it is possible that it works well on skin providing it is not toxic. Another

benefit of using this dye is that after the collection of the dyed cellular material, a full forensic DNA profile can be obtained even if excessive spray occurs which implies that the makers of the DD solution made sure to utilise a dye that does not inhibit the DNA when extraction and profiling techniques are utilised. [37] However the determination by the Young and Linacre (37) study found to be able to observe full DNA profiles after excessive spraying in the single spray with the continuous spray still providing a full DNA profile however it has a negative impact in the levels of RFU units however it was still higher than when the values were compared with the light spray.

Saliva consists of water, electrolytes, a glycoprotein(mucin), inorganic components and buccal epithelial cells. [36] In the liquid form of saliva, DNA is not present however it is present in the cellular material that is sloughed from the inner lining of the mouth such as the epithelial and glandular cells which are added into the saliva via their natural shedding. [17] The DNA in saliva has been found and analysed from a variety of possible trace DNA evidence types such as stamps, envelope flaps, cigarette butts, drinking vessels, masks, foodstuffs and the samples from a sexual assault victim's body. [36] Now this last one is important to the forensic examiners as it has been determined that wet biological material transfers much more readily than dry biological material and thus increased levels of DNA can be found from saliva than from touched objects, however this is heavily contested. [3, 21, 35-41]

Blood is usually the preferred biological fluid for the DNA found to be used for a DNA profile, saliva can also contain a high amount of DNA quantity with the degradation of this DNA being minimal to none at best. [6] In addition to this a study by Kenna et al. (17) found that saliva can stay on skin for more than 96 hours with the donor providing a fully successful DNA profile. However, it must be remembered that a victim's hygiene plays a large role and as sexual assault is usually very traumatic on a variety of levels, showering after a sexual assault is quite normal. [3,7,36] This is the reason for sexual assault victims to instinctively desire a shower immediately after an assault and why they are usually encouraged universally to go to a sexual assault resource centre to get checked out as the shower allows for all the forensic trace evidence to be washed away by the water. [3,7,36] However a study by Williams et al. (6) determined that even after showering, the salivary DNA is retained by the skin and thus in 60% of their results the male DNA profile can be achieved. As previously discussed the largest problem in obtaining this saliva trace evidence is locating it and this could potentially be achieved by the dye discussed earlier and any injury located on the victim's body can be potentially helpful in isolating the location of the saliva deposit. [14] Another pitfall in locating where the invisible salivary DNA was deposited on the victim is that they unfortunately cannot definitely remember the entirety of what happened before, during and after the sexual assault usually a consequence of either alcohol and/or drug consumption. [11] Another potential solution for this is the presumptive testing for amylase to determine the estimated location where the saliva was deposited before sampling. [11] What must be remembered by the forensic examiner is that when sampling the victim, it is imperative to avoid any secondary victimisation by not conducting any sampling unnecessarily. [11] Thus when choosing a possible sampling technique, it is imperative to consider potential occurrence of any secondary victimisation in addition to the cost and practicality of the chosen technique. One additional factor that impacts trace DNA evidence collection is that public awareness of how helpful DNA is in criminal investigations has never been higher and thus actions have been taken by the offender to minimise this evidence

such as them wearing a condom and in addition to this there is an increased amount of information being requested by the courts such as determining if any traces of condom lubricants are present on the intimate swab sample. [13]

2.3 Swabs as a sampling method

Swabs have been used forensic sampling of a variety of different surface types for the collection of trace DNA evidence for many years and is the most common sampling technique for forensic use. [42] These swabs are usually used as they act as the intermediary between the trace DNA evidence collected from the object and then they are retained for the time being until the time for the forensic examiner to begin processing and analysing the evidence. [43] Hence why it is so important for the sampling technique chosen to be performed correctly as if there is no biological evidence on the swab nothing conclusive or otherwise would be produced. [10] However there are no universally agreed method for the application of the swab across the victim's body or the crime scene. As it is impossible for the same exact amount of force and motion to be used by the same examiner each and every time they conduct a swabbing, there are some guidelines which are generally agreed on. Such as if the surface the swab is targeting is dry then the swab should be moistened before sampling, because otherwise inefficient sampling would occur as a consequence of dry swabs being unable to solubilise the dry cellular material. [3, 42] Also when using skin as the surface sampled from, it is generally agreed that the swab should be rotated confirming that each part of the swab comes into contact with the target surface. [6, 31, 44] Predominantly in the past, when sampling for trace DNA using swabs, a single moisture swab is utilised however nowadays the common sampling technique is double swabbing where a moistened swab is then followed by a dry swab. [1,14, 36] However there are a variety of swabs available each of which differ by either the design, size and shape and therefore the correct swab for the purpose and must be carefully selected depending on what the examiner is aiming for. [3]

2.3.1 Swab types

The effectiveness of a certain swab type can depend on a variety of different factors but it is argued that the most imperative factors are its composition and design. [18] Swabs can be typically classified into three different categories such as wound swabs, flocked swabs or pad swabs. [15, 18] The most commonly used swabs are the cotton or rayon swabs and they are part of the wound swab category due to the method of how the fibre or many fibres are wound around the shaft of the swab creating a bud. [15, 18, 45] Research relating to these cotton or rayon swabs illustrates that due to its tightly wound bud of fibre/s they contain a limited surface area for the collection of the trace biological evidence. [42] An alternative to this is the flocked swab where the swab is created by multiple short nylon strands that have been directly glued to the shaft pointing outwards causing the swab to have an increased surface area when compared to the rayon or cotton swab. [40,43-44] Finally foam swabs are a type of pad swabs where they incorporate a sleeve of foam connected to the end of the shaft. [43-44] It has been proposed that the foam matrix allows for the swab to possess a more flexible nature which provides a better penetration into the surface being swabbed. [43] However, unfortunately there is no clear agreement throughout the research about which swab type is the most effective as it appears the results to differ between the surface used and what type of biological material is being collected.

The decision on which swab type to use to collect the cellular material is usually impacted by both the price and the convenience. [31] One study conducted by Verdon et al. (18) determined that certain swab types work much more effectively for certain biological evidence types from different substrates. It was found that foam swabs were outperforming in biological material obtained by each of the tested swab types in regards to wood and flocked swabs were outperformed by the other swab types for both the substrate tested and which biological material obtained. [43] One surface that the Verdon et al. (43) study did not test was skin and the increased amount of efficiency in sampling did not always constitute into increased extraction efficiency. As the sampling techniques for biological material have to be able to release the biological material gathered onto the sample in order for a successful extraction for DNA profiling to occur. The manufacturers of the flocked swab type claim that its design allows for increased absorption and release rate and in the Brownlow et al. (44) study it was found that it was potentially a useful tool in a controlled laboratory but using these at a crime scene where conditions are not uniform and unexpected factors come into play is potentially another story. In contradicting research, a study by Brownlow et al. (44) found that flocked swabs were not more effective than cotton swabs with both capable of obtaining and releasing a considerably high amount of DNA. In comparison to this an earlier study by Dalmaso et al. (46) found that flocked swabs had an instantaneous release rate of 80% of the absorbed material obtained when sampling. However, there is no guarantee that the one swab will collect all the potential DNA trace evidence present at a given location and thus why double swabbing is now the most commonly utilised sampling technique in this field. [1, 24, 31]

2.3.2 Double swabbing

Double swabbing is a technique consisting of first using a moistened swab on the assumed area on the victim's body or object found at the scene of the crime and then after the moistened swab, a dry swab is then administered to the same area. [1,14,36,42] After this occurs, both the moistened and the dry swabs are then utilised in joint extraction and thus allowing for the greatest amount of DNA trace evidence to be collected increasing the chances of a full DNA profile being obtained. [1,42] The use of the moistened and dry swabs after each other has been postulated that this methodology should be used like this as it allows for any loose cells the moistened swab unveiled through its use, the dry swab would be able to secure them. [22] However, studies have been questioning this facet of the double swabbing technique where more cellular material is able to be obtained by the dry swab after the moistened swab. Such as a study by Pang and Cheung (44) which tried to determine whether the dry swab increases the amount of cellular material obtained by the double swabbing method by extracting the amount of DNA from the swabs independently of each other. This study (42) found that the amount obtained from the dry swab still contained enough DNA to obtain a DNA profile and thus from this the conclusion that using the double swabbing method to increase the amount of possible DNA obtained from cellular material left behind by the offender on the victim's body or on items found at the scene. However, it must be remembered that this study by Pang and Cheung (42) conducted the study using laboratories where the conditions were constant and not changing unlike the real world in addition to this they also used trace DNA obtained from surfaces where high traffic occur such as light switches or door handles hence they did not use skin as a surface.

The original recommendation for first using double swabbing was in 1997 by a study by Sweet et al. (24) to use the double swabbing technique collection of the cellular material of saliva from skin. However, the skin surfaces the Sweet et al. (16) study utilised for the research were laboratory based cadavers which meant all conditions utilised in the study were constant and thus possibly decreasing the likelihood of external factors such as clothes and the existence of background DNA impacting the results. Hence the reason why recent research of this type being done on living participants skin and thus why results have been contradictory.

A study into the effect single and double swabbing techniques have on the collection of semen on skin by Ferreira-Silva et al.[1] found that there was no statistically significant difference between either technique in the quality of the DNA profiles produced in addition to the quantity of DNA collected. As the Ferreira-Silva et al.[1] study was working under the assumption that semen would react to the moistened swab followed by the dry swab like the skin cells mentioned previously (DNA cellular material) however the difference in the type of biological material allows for the alternative hypothesis that the first moistened swab would absorb all the moisture present on the skin and thus nothing would be available to be collected by the dry swab and thus both sampling techniques would obtain similar results. Therefore from these results, Ferreira-Silva et al.[1] reasoned that the use of the second swab maintained no beneficial factors such as an increased in DNA collection whilst increasing cost via the fact that two swabs are needed for the double swabbing technique when only using ione can collect a similar amount of DNA cellular material. However a possible reason for this is that each swab commercially available has different materials forming the tip of the swab. [47] Each separate tip material that is available for the use in this field would also have different chemical and physical characteristics which may impact the rate at which the DNA cellular material absorbed by the swab is both collected and released which impacts how successful the DNA profile developed is.[47] The research relating to this double swabbing method recommends this method to be utilised in regards to the collection of trace DNA from a variety of different substrates however there are far less studies focussing on how the different sampling methods compare with each other.[37, 44, 48]

One study from de Bruin et al.[22] that actually did compare double swabbing with the trace DNA technique tape lifting with the method utilised being stubbing found that when collecting the offender DNA, both techniques utilised performed well yet the results in regards to the DNA profile made from the collected trace DNA were produced quite similarly in regards to both having the same peak height and same number of alleles found. [14] However the de Bruin et al.[14] study did find a minute difference in the amount of victim trace DNA evidence collected by the double swabbing method causing a greater offender to victim ratio. In sexual assault cases, the assailants' DNA is obtained by them leaving behind their cellular material on the victim's skin or on objects left at the scene, as it is impossible to avoid obtaining the victim's DNA, in order to minimise the risk of this having a disruptive impact on obtaining a successful DNA profile, the ratio between offender DNA and victim DNA should be as high as reasonably possible. [14] It is possible that these results illustrated by the de Bruin [14] study could be obtained due to the difference between the two methods contact type as in regards to the swab, little contact force is required due to the swab and surface having minimal contact needed and therefore it is possible that it decreases the amount of trace victim DNA observed and analysed.[14] Further research in the

determination of whether this theory is supported or disproved is required as it must be considered that each individual uses a different amount of force they use per sample and numerous studies[37, 44, 48] have used this double swabbing technique on different substrates however controversy still exists around whether double swabbing is the superior sampling technique.

2.4 Tape-lift as a sampling method

Another sampling methodology that is well established in the field of obtaining the trace DNA evidence is called tape lifting.[49] Presently adhesive tapes can be utilised for a wide variety of different evidence types, with textiles being the most primary.[50, 51] This method is getting increased utility as a result of it being more easily accessible for its ability to both sample large evidence types and whilst collecting the trace evidence, it does not damage the evidence it is used on.[49, 52] It has been found to be used for the collection of epithelial cells porous surfaces and inappropriate for use in wet or damp locations.[53, 54] A nice consequence of the adhesive nature of tape is that it does not require any moistening agent to be used and thus there is no time needed to be allocated for drying and also no obvious complications with any possible water soluble impurity present in the sample. [17] This method of sampling is considered to be considerably fast and simple to utilise and is considered to be quite cost-effective.[40]

The trace DNA is transferred to the tape when present on a surface by being certain that the adhesive side of the tape is facing down onto the surface to be sampled and then by continuously pressing the dry non-sticky side allowing for the collection of the trace DNA to be adhered to the adhesive side of the tape.[36] The normal traditional method of tape lifting is usually conducted by the forensic examiner using their gloved hands to place tape around their fingers.[22] However it is believed by researchers in this field that this could be a potential risk of contamination and thus a new methodology for tape lifting was formed called stubbing which is where the double sided adhesive tape is wrapped around a scanning electron microscope stub instead of the forensic examiner's gloved fingers, allowing for a reduced risk of contaminating as the researcher is now further away from the evidence being tested.[22, 54]

The methodology behind stubbing is the same as tape lifting however with the adhesive part of the tape being pressed onto the tested surface until all of the adhesive is full and cannot get more cellular material on the tape.[22, 51] The direct extraction of the tapes is preferred over swabbing the tapes as it was found that swabbing the tape samples is quite tedious , time consuming and did not result in a complete retrieval of all the present trace evidence from the tape. [51] A study by Barash et al.[55] found that from variety of the commercially available tapes on the market only some are potentially useful for both extraction and collection of the DNA material. Unfortunately, there has not been an abundance of research in regards to determining which DNA extraction method works the best with specific types of tape.[51]

A large prevalent amount of the research in the use of tape lifting has been related to its use in textile sampling and not skin. A study by Albujja et al.[56] recommended that using the tape lift methodology would be a much less intrusive alternate method of collecting the trace evidence. In this study by Albujja et al.[56] two types of adhesive tapes were compared to the single swab methodology to determine which method developed the higher levels of DNA from the epithelial cells left behind. This study [56] found that there was no difference

between the traditional tape lifting method and the single swab however it was a valuable proof of concept of obtaining epithelial cells from skin.
However a study by de Bruin et al. [57] concentrated more on the collection of offender DNA from a victim's skin and they did this by comparing the stubbing method to the double swab technique. They found that the stubbing method collected more DNA it also contained higher levels of the victim's DNA also.[22] To obtain a successful informative DNA profile, it must contain minimal background DNA and an abundance of the desired offender DNA.[54] This could occur because of the discrepancy between the small amount of force used for a swab while for the tape lifting method, the tape must be dabbed onto the skin until no longer adhesive, therefore increasing the amount of victim DNA observed.[14,54] Contrary to this, it has been found that the saturated tape would contain much more cellular material when compared to the swab and thus the offender DNA is a limiting factor.[14] Thus if there has been more offender DNA present on the victim's skin it would be collected by the tape lift causing an increased ratio between offender DNA and victim DNA.[14] However as in the case of sexual assault crimes, the amount of DNA left behind on the victim is minuscule and thus the potential use of tape lifting is not an ideal methodology and thus the use of mini-tape could be more potentially useful since they are both smaller and are pressed not dabbed onto a surface.

2.4.1 Mini-tape

Another type of tape lifting methodology is the mini tape lifting method which has been shown to be both reliable and easy to utilise for the sampling of DNA from textiles.[21, 58] Mini tapes are similar to the traditional double sided tapes used in tape lifting as they are also double sided and instead of being dabbed and they are pressed repeatedly across the substrate.[17] This mini tape methodology is commonly used by forensic examiners in either the lab or at the scene of the crime in order to both collect and preserve this cellular material. [13] Whilst the well-known superiority of the tape lifting method on textiles, as stated previously the use of this tape lifting method on skin is still full of unknown due to a lack of research. One of the small pieces of research in this field is a study by Kenna et al [36] and they have compared the mini tape method to other sampling techniques. The results from the Kenna et al. [36] study is that mini tapes work much more effectively than swabbing, when trying to recover salivary DNA from skin and they also determined that the use of this adhesive tape is a lot faster due to the lack of drying time needed. Hence why there is further research into these mini tapes required as they could be a potential improved sampling method. There has not been a whole lot of research into determining the effectiveness of a sampling technique against other sampling techniques and thus hopefully this mini tape method is the best of both tape lifting and swabbing in that it is quick and easy to use like a tape lift and can also yield a far greater victim DNA to offender DNA ratio like a swab.

2.5 Alcohol wipes/solution

When collecting the DNA trace evidence from an object by using the swabbing sampling technique, the moistening agent used is usually sterile water.[27] A range of studies have compared this sterile water to other detergent-based swabbing solutions and this detergent-based swabbing solution has been found to exceed the sterile water.[27, 59] However in spite of the lack of research into whether sterile water is the most superior solution for the collection of this cellular material, it is still the most commonly used solution.

A possible alternative solution to this is alcohol-based solutions such as using ethanol as the moistening agent, as ethanol is a material that is easy to access and due to it being a weak acid allows for its use on a multitude of surfaces without damage.[60] However it must be remembered that ethanol is an acid albeit a weak acid, if any small injuries are present on the victim this will cause a mild distress due to the acid causing discomfort but this is a case by case basis as each different person will have an unique reaction to this similar to going to the beach with an open wound.

Ethanol is typically utilised in this field as a fixative to preserve post-mortem tissue specimens since it has been found to decrease the degradation of DNA due to its natural antibacterial abilities.[56, 60, 61] As the use of a 70% alcohol solution has the benefit of allowing for microbial growth to be either delayed or even prevented for up to 5 days in less than ideal storage conditions.[60] As the swabs moistened with sterile water need to be dried out in order to store them safely in order to prevent the degradation of the DNA, if an ethanol based moistening solution was used it would hasten the amount of time needed for the swab to be dried due to its small evaporation time.[60] However, in spite of alcohols' potential benefits as a swab moistening agent, rarely has any research been undertaken in regards to its ability at obtaining the trace evidence from any substrate not even skin, the nearest thing related to this is the self-moistening swab referred to as a mini-popule.

The mini-popules' swab handle contains an ampoule which contains a solution of 91% isopropyl alcohol which is how these swabs self-moisten.[18] The use of mini-popules in research is somewhat minimal, one study conducted by Verdon et al. [43] found that the self-moistening foam swab performed quite poorly frequently coming last in the efficiency rankings in a variety of substrates. Verdon et al. [43] then theorised that as the isopropyl alcohol contained a lesser solubility when compared to water, the solution's interaction with the trace DNA evidence had decreased efficiency levels. While these results regarding isopropyl alcohol were not promising, a study by Harris [23] put forward an alternative sampling method using 70% isopropyl alcohol wipes as they are relatively easy to access, cheap and they were also already moistened. If the research is positive, then this method of sampling could be a simple and easy method for the forensic examiner to perform in addition to the wipe being available for storage without being dried out. This alcohol wipe sampling technique showed promising signs in the Harris [15] pilot study yet more research is needed for validity and reliability. Early evidence kits in relation to sexual assault are advocated to both preserve the much needed trace DNA evidence when the victim is delayed in accessing a forensic examiner for samples to be taken. [10] It also acts to potentially decrease the amount of trauma the victim has as a result of the sexual assault and if an alcohol wipe was utilised it could potentially decrease the chance of secondary victimisation. [11,15]

3.0 EXPERIMENTAL DESIGN

3.1 Sample collection

Five independent pairs will be sampled for two mock scene scenarios where one male would be playing the role of the offender and one female playing the role of the victim.

3.1.1 Simulated detaining of the victim via a wrist grab

The offender is to grasp the wrist of the victim for a 30 second time period. To simulate a mock assault, the offender is to use a firm grip, without causing pain. Simultaneously, the victim rotates their wrist externally and internally 180 degrees to simulate restraint. The

victim is then to go about their everyday activities, avoiding unnecessary contact with the area for 6 hours before being sampled. Two replicates per pairing will be sampled each day by one of the following collection methods:

- Single foam swab
- Double swabbing
- Scotch tape-lift
- Mini-tape
- Alcohol wipe

3.1.2 Simulated slobber/kissing on victims' neck

Preliminary analysis will be performed to determine the approximate volume and cell count per µl in a replicated 'lick'. From these five different stock solutions will be made from each of the five male volunteers DNA from each set of independent partners. Using the stock solution, the predetermined volume of buccal rinse('lick') from the offender' stock solution will then be pipetted onto the neck of the female victim and allowed to dry. The victim is then to go about their normal everyday activities while avoiding unnecessary contact with the area for 6 hours before being sampled. Two replicates per pairing will be sampled each day by one of the following collection methods:

- Single foam swab
- Double swabbing
- Scotch tape-lift
- Mini-tape
- Alcohol wipes

3.2 DNA extraction and quantification

All DNA from samples will be extracted using the Qiagen extraction method and quantified using a Quantifiler Trio. Using this information to produce a ratio of male to female for comparison purposes. The average total male DNA yielded will then be calculated and compared for each tested mock scenario. After this, the appropriate statistical test would be then utilised to determine how significant these results are.

3.3 Data analysis

The data output from the Quantifiler Trio will be analysed to determine the total DNA quantification and the offender DNA concentration(Y-DNA) yielded from each sample. This information will then be used to create a ratio for comparison between male and female DNA. Average total male DNA yielded for each sampling method will be calculated and compared for each mock assault scenario. An One way t-test statistical test will be utilised to determine whether the difference between samples is statistically significant.

4.0 EXPERIMENTAL AIMS AND HYPOTHESIS

This study aims to determine which sampling technique is the most effective in recovering the male offender DNA from the female victims' neck/wrist skin. The pre-existing sampling methods that will be analysed in this study for the recovery of male offender DNA from the skin of the female victims' neck/wrist is the single foam swab, double swab, mini-tape, tape lift and alcohol wipes (potential recovery method used in the previous study). This study aims to determine the most effective sampling technique for the recovery of the male offender cellular material from the female victim skin.

1) Evaluate which sampling technique yields the largest concentration of male(offender) DNA, given a mock assault where skin to skin contact occurs.

2) Evaluate which sampling technique yields the largest concentration of male(offender) DNA, given a mock assault where offender saliva to victim skin occurs.

4.1 Experimental hypothesis 1

H_1: The greatest yield of offender DNA recovered from a female victim's skin, six hours after a simulated mock assault (where a predetermined amount of buccal cell stock solution from a male offender was pipetted onto the neck of the female victim), would be achieved by the use of the double swab technique when compared to the other tested sampling techniques.

4.2 Experimental hypothesis 2

H_1: After a simulated mock assault where the male offender grabbed the wrist of a female victim, the scotch tape lift will yield the largest amount of offender male DNA from the female victim's DNA when compared to the other tested sampling techniques.

5.0 CONCLUSION

In sexual assault cases it is quite often that the sole crime scene available for evidence collection is the victim's body. The likelihood of a successful DNA profile being produced is quite heavily impacted on whether the sampling method conducted was conducted properly with minimal contaminants obtained and thus there is cellular material present in the sample. There are multiple different factors that influence how well the sampling and extraction methods go such as background DNA, shedder status and washing. Contrary to the contradictory results of the studies in this area it is usually agreed that for a sampling technique to be at its most effective, a ratio of increased offender DNA to decreased victim DNA as possible is desired. From this it can be argued that swabbing is more effective than tape lifting however the research where this was all formalised with a coherent and clear result of which sampling method is the most effective does not exist. Some studies exist where the single swab is compared to the double swab and tape lifting is compared to both stubbing and mini tape lifting however there is no study apart from the Harris (15) study where most of the sampling techniques are looked at and compared to each other as a whole. This study is aiming to obtain this much needed clear and concise conclusion on which sampling technique is the most effective however there has to be more research in this field to both validate and allow for these results to be reliable also.

6.0 Reference List

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Part Two

Manuscript

Sampling techniques for the recovery of male offender DNA from the female victim skin surfaces

Masters in Forensic Science Literature Review

Sampling techniques for the recovery of male offender DNA from the female victim skin surfaces

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Abstract

Sexual assault is a very serious criminal offence with great health and welfare repercussions. Trace DNA is often referred to as the minute quantities of DNA that can be transferred between a victim, perpetrator and/or the crime scene during an assault. Often in a sexual assault case the victim's body is the most important crime scene and sometimes the only available crime scene. Because of this it is critically important to successfully take a DNA sample after the assault. There has only been one unpublished study that has touched on the comparison of all available sampling techniques. This study aimed to compare and analyse five sampling techniques consisting of double swabbing, single swabbing, tape-lifting, mini tape-lifting and alcohol wipes which was a somewhat new method. To do this, a scenario where saliva from the male offender is found on the female victim's neck was conducted for this study.

While the results were considerably contaminated, it was found that double swabbing obtained the highest DNA concentration of samples when compared to the other four methods. Also, these contaminated results also provided somewhat promising results in the use of tape-lifting, mini tape-lifting and alcohol wipes. But these methodologies would need to be verified via future peer reviewed research as these contaminated results were unhelpful in providing an idea of what these results were but cannot conclusively support any of the hypotheses provided. Overall, this study did not find anything ground breaking due to the largely contaminated results of this study, but it is imperative that follow-up research takes place to provide verification that while these results were contaminated something of value may have been found. Unfortunately, nothing of any statistical significance was found due to the contamination and hence in the future great care when moving samples from tube to the well plate should be taken to minimise the risk of contamination.

1.0 INTRODUCTION

Sexual assault is a severe public health problem affecting millions of people worldwide, particularly women, and is regarded as one of the most heinous type of crime due to the amount of extreme psychological, physical and emotional trauma that often impacts the victim long after it occurs.[1, 2] Sexual violence is defined by the World Health Organisation (WHO) as any sexual act, attempt to obtain a sexual act, unwanted sexual comments or advances, or acts to traffic, or otherwise directed against a person's sexuality, using coercion, by any person, regardless of their relationship to the victim in any setting, including but not limited to home and work.[3] This is relevant as one subcategory of sexual violence is sexual assault, and this is defined by the WHO as the act of using physical or any other type of force to obtain or attempt sexual penetration.[3] One type of sexual assault is rape, defined as the physically forced or otherwise coerced penetration of the vulva or anus with a penis, other body part, or object, although the legal definition of rape may vary and, in some cases, may also include oral penetration.[3]

In Australia, it is estimated that 17% of all women aged over 18 and 4% of all men aged over 18 have experienced sexual assault since the age of 15.[2, 4] It has been found that women would be most frequently sexually assaulted by friends or acquaintances of the victim

followed by intimate partners and then strangers, respectively.[5] Trace DNA is often referred to as the minute quantities of DNA that can be transferred between a victim, perpetrator and/or the crime scene during an assault and this type of evidence is what this study is focusing on.[5]

Often in a sexual assault case the victim's body is the most important crime scene and sometimes the only available crime scene.[5] This biological evidence left behind by the offender during the act of sexual assault is absolutely critical because it can potentially place the offender at the scene of the crime[6], provides corroborating evidence that contact has occurred between the victim and the offender[5, 7] and essentially help with the elimination and identification of persons of interest.[7] From this a reasonable conclusion to reach is that it is critically important that in cases of alleged assault, trace evidence must be collected correctly and examined as soon as reasonably possible. This is because after a sexual assault has occurred, many of the victims of sexual assault have a shower or bathe, possibly washing most if not all the trace evidence off.[8] Another potential pitfall is whether the victim of the sexual assault can definitively remember the locations on their body that the offender touched them, this is a problem as with swabs the examiner must be swabbing in a location that has the male saliva (trace evidence) in order to obtain the supporting trace evidence of the assault.[8]

The successful collection and recovery of the DNA trace evidence belonging to the offender from the body of the victim of sexual assault is most impacted by the selection of an appropriate sampling technique.[9] There are several methodologies available for the collection of cellular material from a multitude of different substrates at the present time, including several swabbing and tape-lifting techniques.

In the field of forensics, one prevalent sampling technique is the use of swabs as they have been used for a variety of different substrate types for many years.[10] There are many different types of swabs where they range in size, shape and design depending on the substrate they are intended for.[5] Swabs can be typically classified into three different categories such as wound swabs, flocked swabs or pad swabs.[11, 12] Cotton swabs are categorized as wound swabs as they contain either one single long fibre, or many different fibres, are tightly round a shaft forming a bud.[11-13] Due to this tightly wound nature of the wound swabs, the surface available to collect the trace evidence is limited. [10] An alternative to the wound swabs is the flocked swabs as they are thought to contain a larger surface area in comparison as they contain short nylon strands that are glued to the shaft and are protruding outwards.[10-12] The last alternative type of swab are the foam swabs which is a type of pad swabs which is where a sleeve of foam is attached to the end of a shaft.[11, 12] However as skin as a substrate has not been conclusively investigated, it is unknown which swab type allows for the collection of the most cellular material and in forensics, traditionally a moistened swab is typically used for the collection of cellular material.[1, 14, 15]

It is impossible to make certain that each individual examiner all over the world uses the exact same type of swab and the exact same amount of force. However, there are some universally agreed upon guidelines such as if the target substrate is dry like skin, then the swab typically needs to be moistened before sampling[5], since dry swabs are not able to solubilize the dry biological material, therefore causing ineffective sampling.[10] Also swabs must be rotated in such a way that each part of the bud comes into contact with the target location.[8, 16, 17] But it is not guaranteed that the single swab will get every piece of available

cellular material on a substrate[1] and as a result of this double swabbing[17] is now a common practice for forensics as first put forward by Sweet et al in 1997.
The double swabbing technique involves the use of two swabs where a moistened swab is first used and a dry unmoistened swab is used after to obtain any of the cellular material that was loosened by the first moistened swab and secured by the dry swab at least in theory. [1, 15, 16, 18] After the use of the two swabs they would then undergo joint extraction.[1, 16] However it has been argued by Newton[7] that if the moistened swab absorbed all the moisture present on the skins' surface, then there would be nothing available for the dry swab to collect. This theory was then argued by Pang and Cheung[16] who found that the second (dry) swab alone was enough DNA to obtain a usable DNA profile therefore it can be theorized that using the double swab method double the amount of cellular material can be potentially obtained. This finding however has been rebutted by Ferreira-Silva et al.[1] where they found that when comparing the two swabbing methods (single and double) nothing of any statistical significance between the amount of DNA found by either method. There are several studies that advocate and use this double swabbing technique that it is the traditional method, there have been no successful peer-reviewed study that illustrates the superiority of double swabbing over the other techniques.
Another well-established sampling technique is the use of adhesive tapes, one method being tape-lifting, a method that was originally utilized for the collection of loose microscopic material such as hair and fibres.[19] Presently, the use of tape-lifting is prevalently utilized in the collection of trace evidence from a variety of different exhibit types but textiles is more prevalent.[20, 21] The traditional tape-lifting methodology is where the examiner wraps tape around their gloved fingers with the adhesive side facing outwards.[18] This tape is then repeatedly dabbed onto the target substrate until the tape is so saturated that it can not fit any more material onto the adhesive tape.[15] As the tape is already adhesive unlike the swabs, no moisturising agent is required, therefore no additional complications involving water-soluble contaminants and no drying time required.[15]
A journal article by de Bruin et al.[18] where they were comparing the amount of cellular material obtained from victim skin using the tape-lifting method with the double swabbing method. This study[18] found that both methods performed equally well with small differences in the amount of victim DNA recovered. As when sampling a victim, it is only possible to minimize the amount of victim DNA obtained as it is impossible to use a sampling method and get no victim DNA whatsoever, to minimize this risk, care should be taken to make sure the ratio of offender DNA to victim DNA is as high as possible.[18] The cause of the increased amount of victim DNA obtained using the tape-lift method is possibly due to the difference in contact time between the sampling technique and skin, because there is an increased amount of contact between the tape and the skin when comparing to the minimal amount of contact occurring using a swab.[18] The use of mini-tapes may potentially be more promising as they are more pressed onto the skin than dabbed like the tape, potentially increasing the amount of victim DNA obtained also. Dabbing is when the examiner repeatedly places the tape on a surface repeatedly until it is clear no more particles can be placed on the tape hence it will stop being sticky.[18] Whereas pressing is when the whole tape is placed on the surface with fingers pressing on the tape to make sure the sample has been taken and no repeated motion is needed unlike the dab.[18]

Mini tapes are one-sided adhesive strips that have been shown to be reliable in obtaining trace evidence from textiles.[9, 22] There have not been all that many studies that have utilized the mini-tape method in the use of obtaining trace evidence from victim's skin, one such study being the Kenna et al.[15] which found that the mini-tape method was generally more effective in obtaining trace evidence than swabbing when recovering saliva from human skin. Unfortunately, there is no study comparing all available sampling techniques using skin as a substrate. Harris[14] tried to do this with the one volunteer however it is likely that not much of any statistical significance was found due to the lack of sample size. A study by de Bruin et al.[18] displayed little to no variation in the methods utilized whereas Sweet et al.[17] found that when using an additional dry swab it was possible to double the amount of cellular material but this has since been contradicted in some studies.

As there is little to no universally agreed upon method that is most favoured in getting cellular material from victim's skin, one new sampling technique that was put forward first by Harris[14] is the use of alcohol wipes as they are easily available for medical and healthcare workers. As they are less invasive and they are relatively cheap, so simple to use that the victim can potentially sample themselves and they are both sterile and pre-moistened both of which are desirable characteristics when theorizing a new sampling technique for obtaining offender DNA from a victim of sexual assault. As alcohol wipes can be stored without the need for drying, they are excellent for early evidence kits. This study is aiming to determine which of the 5 sampling techniques chosen obtained the most DNA from the victim's neck as it is important to identify and validate which sampling technique is the best practice for sampling evidence after a sexual assault. The hypothesis this study was operating on was that double swabbing would obtain the greatest amount of DNA and that alcohol wipes would obtain a good amount of DNA from a victim after sexual assault.

2.0 MATERIALS AND METHODS

2.1 Mock Assault Design

For this study there was 4 female volunteers and 1 male volunteer with male volunteer donating 12ml of saliva. The male volunteer was acting in the role of 'offender' while the female volunteers were acting in the role of the 'victim'.

2.1.1 Scenario 1: Slobber/Licking on Victim's Neck

Before starting the experiment, a cell count of the male saliva that was donated by the male volunteer was undertaken using the Biorad TC20 Automated Cell Counter and it was found that there were 7.25×10^6 cells/ml with the living cells being 1.30×10^6 cells/ml. As a result a dilution of the saliva was required and according to a study by Cahill and Chapman (23) 20000 cells at least were needed to obtain an usable DNA profile. From this a 1 in 10 dilution of the saliva samples was created to increase the 2.8571µl of the undiluted solution into a much more manageable amount which was 29µl of the diluted saliva solution. This diluted stock solution amount was then pipetted onto both sides of the female volunteers' neck. Allowed to dry using a mechanized fan and then the female volunteer was told to go about their usual everyday activities for 6 hours, avoiding direct contact to the area and instructed to not wash. After the elapsed 6 hours, one of the five sampling techniques were utilised to take the sample.

2.1.2 Sampling

Samples were taken from the skin of the female volunteer, from both sides of their neck. This process was then conducted, when the female volunteers were free for 5 days to allow for the use of the five different sampling techniques.

2.1.3.1 Double Swabbing

The double swabbing technique was performed using two sterile rayon-tipped swabs. As per the method outlined in Sweet et al. [17], the target area where the saliva sample was deposited would be swabbed by first a wet swab (moistened with sterile water) and then followed by a dry swab. The swab head was then rotated to allow for the entire head of the swab to be in contact with the target area. Swabs were then returned to their sterile protective case, labelled and stored in a freezer at -20°C until extraction.

2.1.3.2 Foam Swabbing

A single sterile rayon-tipped swabs was first moistened with sterile water. The target area was then swabbed with the swab head being rotated to allow for the entire head of the swab to be in contact with the target area. Swabs were then returned to their sterile protective case, labelled and stored in a freezer at -20°C until extraction.

2.1.3.3 Scotch Tape-Lift

A new roll of scotch tape was used, with the first metre of the tape being discarded to minimise contamination. A length of approximately 20cm of tape was then wrapped around the end of three double gloved fingers, with the adhesive side facing out. One side of the adhesive tape was then repeatedly dabbed onto the target surface until the tape was no longer adhesive. Samples were then placed into individual petri dishes, labelled and stored in a freezer at -20°C until extraction.

2.1.3.4 Mini-Tape

A Scenesafe FAST™ tape was used to perform the mini-tape method. Holding the non-adhesive end of the mini-tape where it was marked with the Scenesafe FAST™ brand, the mini-tape was then removed from its backing paper. The target surface area was then sampled by placing downward pressure on the back of the mini-tape and lifting repeatedly, until the mini-tape was no longer adhesive. Samples were then placed into individual petri dishes, labelled and stored in a freezer at -20°C until extraction.

2.1.3.5 Alcohol Wipe

The alcohol wipes used were wipes saturated with 70% v/v Isopropyl Alcohol was removed from packaging and unfolded. One side of the alcohol wipe was then repeatedly wiped across the entirety of the target surface. The alcohol wipe samples would then be placed into individual petri dishes with the sample side of the alcohol wipe facing down, labelled and stored in a freezer at -20°C until extraction.

2.2 DNA Extraction

Extraction of DNA using the QIAamp®DNA Investigator Kit was performed as per the manufacturer's handbook following the protocol outlined for the Isolation of Total DNA from Surface and Buccal Swabs. The total volume of Buffer AL added to each sample was 400 µl,

and the total volume of Buffer ATE was 40 µl. After extraction, all samples were labelled and stored in a freezer at -20°C. Carrier RNA was used to increase the likelihood of binding occurring.

2.3 DNA Quantification

The QuantStudio®6flex qPCR Instrument was set up following the manufacturers guidelines. Standards and reactions were prepared as per the Quantifiler™ HP DNA Quantification user guide in a 384 well with 20 µl reactions. However due to time constraints the samples were placed overnight in a 96 well plate and the samples had to be placed into a 384 well plate prior to being placed into the QuantStudio®6flex qPCR Instrument. All samples were thawed, vortexed and centrifuged before being added to their respective wells.

2.4 Data Analysis

The Quantifiler® HP data output allows for the quantitative and qualitative assessment of total human DNA. The data output attained from the small amplicon signal represents total human DNA detected for each sample. The average concentration was calculated and compared for each sampling method. A one-way ANOVA was performed to determine if there was any statistically significant difference between the techniques. The one way ANOVA was chosen as there were more than 2 groups of samples hence more than 2 means.

3.0 RESULTS AND DISCUSSION

In the past research involving the comparison of sample collection techniques, rarely has any focus been on human skin as a substrate with only Harris[14] being the only other comparative study assessing all possible techniques. The five sampling techniques that were used in this study to be compared and analysed were single swabbing, double swabbing, tape lift, mini tape-lift and alcohol wipes. The scenario that was used for the investigation to simulate a sexual assault resulting in the saliva being located on the female victim's neck. There were no reference samples taken by the male and female volunteers as due to time constraints only a Quantifiler™ HP qPCR kit was used allowing for just the quantification of DNA and not the determination of how much male DNA was found in each sample. The data used for the comparison of each method being the small amplicon of each sample. However, as the samples were completely contaminated it is impossible to determine the accuracy of the amplification efficiency as the IPC of the samples which acts as an internal DNA standard half of the samples showed that the IPC quantity was 0 essentially meaning that when the PCR plate overflowed it either allowed for the IPC to not be used for the calibration or there was no signal that the IPC underwent its positive DNA control for the instrument or it was placed into wells that did not get calibrated for the QuantStudio®6flex qPCR instrument causing the IPC to not be read.

3.1 Scenario 1: Offender and Victim DNA Concentration

Ideally, the optimal sampling technique would obtain the most amount of offender DNA possible when sampling the skin of the victim however due to time constraints, only the Quantifiler™ HP kit was used and as it can only yield the amount of DNA obtained by the sample.[18] Therefore it is impossible to isolate how much male DNA is present in the sample. Hence the Quantifiler®Trio quantification kit would be far more helpful as it allows for the isolation of both how much DNA is present in the samples in addition to how much male and

female DNA is present in each sample. In this study the offender was playing the part of a male with the victim playing the part of a female hence why the differentiation provided by the Quantifiler®Trio would have been more ideal for this study, however future studies can take this under advisement.

As the 384 well plate that was used for the qPCR resulted in a large amount of contamination occurring, nothing of any significance was found when using a One-way ANOVA test as the p-value found was 0.5 which is essentially insignificant. This was most likely caused by the contamination of the 384 well plate while pipetting the samples in preparation of the qPCR running however leaving the 96 well plate in the fridge at 4°C overnight and then transferring the samples to a 384 well plate just prior to conducting the qPCR would also have contributed to the possibility of contamination occurring. As there were four female volunteers with one male volunteer with two samples being taken for each sampling technique it is possible that 8 samples of each different sampling technique may have been too small for something of any significance to be found but this is uncertain and a possibility for future research to overcome this limitation but another helpful limitation for future studies to work on is the completion of the qPCR using Quantifiler®Trio with no contamination occurring.

Of the five sampling methods utilised, the double swabbing technique obtained the most amount of DNA concentration with the single swabbing technique coming last in the amount of DNA concentration obtained by the technique (Figure 1). However, since the samples in the 384 well plate overflowed both indicating that firstly more than the 20µl reactions made for the samples was used. Also, that whilst the samples were overflowing part of one sample may have flowed into different wells causing the DNA concentration of the samples found to be a combination of more than one sample making anything of any significance found mute.

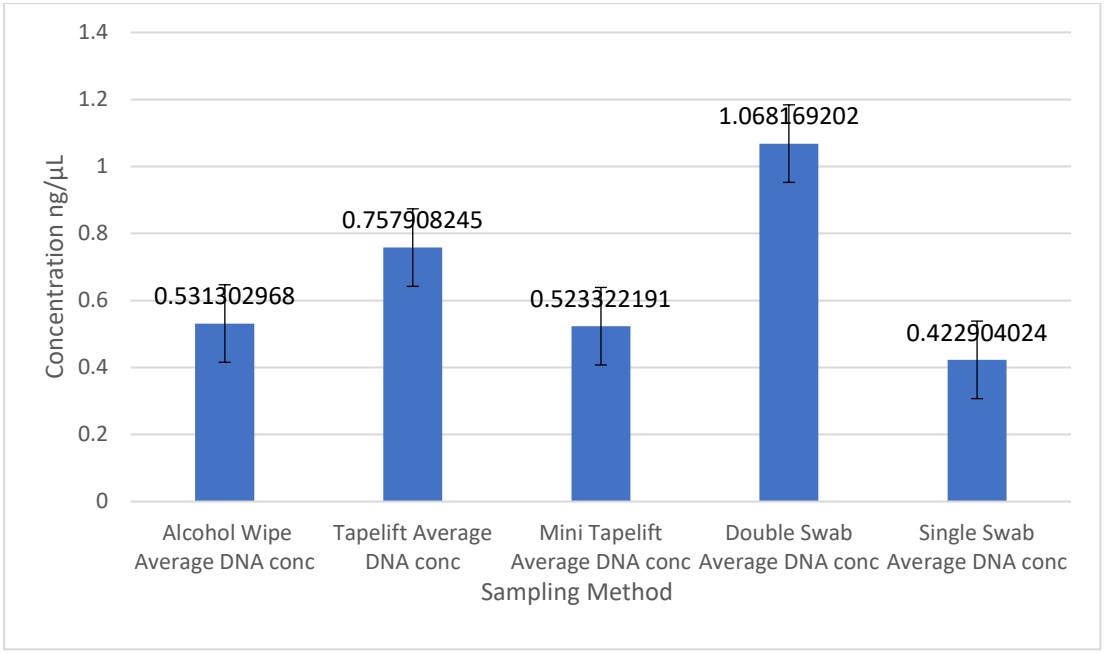

Figure 1. Average concentration (ng/µl) of DNA recovered from victim's skin using each sampling technique after the simulated slobber/licking of victim's neck

It can be determined in isolation when not considering the fact that these results from Figure 1 were massively contaminated that double swabbing method obtained 1.068ng/µl of DNA from the saliva located on the victim's neck (Figure 1). The second highest concentration of average DNA being 0.7579ng/µl from the tape-lift method and lastly the mini tape-lift, single swab and alcohol wipe techniques all obtaining average DNA concentrations between 0.5313 and 0.4229ng/µl (Figure 1). These results are quite satisfactory as the double swabbing technique is considered the favourite for the recovery of offender DNA after a sexual assault when not considering the considerable amount of contamination.[18]
Also when not considering how contaminated the results were it found that double swabbing did obtain more DNA than any of the other four methods which may when support the theory put forward by Sweet et al.[17] where the dry swab allows for the retrieval of any cellular material loosened by the moistened swab allowing for double the amount of DNA obtained when compared to the single swabbing technique. As there is no way of resampling a victim of sexual assault if the first sample taken found nothing. It is critically important that the swab type of preference is also the swab type that provides the most amount of cellular material retrieval. Therefore, the possible research avenues of which swab type is the most efficient would be quite helpful in the future providing minimal contamination of results. Another possible avenue providing time allows would be to use the samples produced and use DNA profiling to determine which sampling technique is both efficient and effective in obtaining enough DNA for a successful DNA profile. One way of this helping future research is that it would be possible to quantify how many donors are present in the sample.
As the tape lifting method obtained the second largest concentration of DNA it is possible that this may be helpful to add to sexual assault kits however it may be invasive and possible that nothing of note was found due to the contamination that occurred with these results. The alcohol wipes technique went decently considering the contamination however more research into using alcohol wipes may be helpful. The alcohol wipe technique did the third best of the five but a universally agreed upon method would be helpful for the future as it was quite easy to be unaware of where on the alcohol wipe the sample took place. Also specifically designed alcohol wipes made for skin may be helpful as when placing the sample into the tube it was quite easy to not know whether the part of the alcohol wipe that sampled the saliva was placed into the tube and not discarded. Perhaps a dye or something that reacts when exposed to human skin may make this alcohol wipe method more effective. The hypothesis that the double swab technique would obtain the highest concentration of DNA was supported by these results if they are taken in isolation without considering how contaminated these results were.

4.0 CONCLUSION

Previously, other than the Harris[14] study no other studies focussed on using human skin as a substrate. While there have been other studies comparing some techniques to each other there has not been a study that incorporated all the available sampling techniques for the use of forensics. With this study five sampling techniques were utilised, and they were compared and analysed, including double swab, single swab, scotch tape-lift, mini-tape and alcohol wipes. The scenario that was used for the investigation to simulate a sexual assault resulting in the saliva being located on the female victim's neck. In future research if desired increasing the sample size may be helpful or increasing the number of replicates for each

volunteer may be helpful. The double swabbing technique was found to recover the most amount of DNA concentration however these results were quite contaminated. Also, these contaminated results also provided somewhat promising results in the use of tape-lifting, mini tape-lifting and alcohol wipes. But these methodologies would need to be verified via future peer reviewed research as these contaminated results were helpful in providing an idea of what these results were but cannot conclusively support any of the hypotheses provided. Overall it was a good pilot study but it is imperative that follow-up research takes place to provide verification that while these results were contaminated something of value may have been found.

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