SAMPLING METHODS FOR THE RECOVERY OF OFFENDER CELLULAR MATERIAL FROM VICTIM SKIN SURFACES

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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: Tiana Harris

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LITERATURE REVIEW

SAMPLING METHODS FOR THE RECOVERY OF OFFENDER CELLULAR MATERIAL FROM VICTIM SKIN SURFACES: LITERATURE REVIEW

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ABSTRACT

Sexual assault can cause major health and welfare issues and is considered a severe and inhuman criminal offence. During an assault, minute quantities of trace DNA can be transferred between a perpetrator, victim and/or crime scene. Transfer of DNA often occurs through strong physical skin-to-skin contact or oral acts resulting in saliva being deposited onto the skin of a victim. Understandably, the skin of a victim can be a critical scene for gathering biological material from the perpetrator to produce a DNA profile, so it is imperative that trace DNA evidence is correctly collected. This literature review aims to address the current research on sampling collection methods available and identify potential problems and factors influencing the recovery of DNA from skin-to-skin or saliva-to-skin contact.

Several factors have been identified to potentially influence the recovery of biological material from skin, including, but not limited to, the effect of background DNA and shedder status. Whether or not an individual consistently deposits the same amount of DNA every time, particularly after washing hands, is still an area of constant debate. Nevertheless, background DNA has been found to impact the conclusiveness of a profile, and while it is unavoidable, a sampling method that collects the least quantity of background DNA would be extremely beneficial. There are several sample collection methods available including the single swab, double swab, tape-lift and mini-tape. Unfortunately, the use of adhesive tapes is more common for the recovery of cellular material from textiles, with little research focusing on skin as a target surface. Presently, the double swabbing method is considered the gold standard technique for sampling skin, with numerous studies utilising this procedure. While the idea behind this technique is promising, the support for this

method was found to be far from universal. There is a lack of overwhelming support for any single sample collection technique, therefore a new method could be introduced using alcohol wipes. Not only are alcohol wipes pre-moistened making for a faster application, but they are also cheap and easily accessible to medical and healthcare workers. There is a need for a single study to compare all the available and potential collection methods, focusing specifically on the recovery of offender DNA from victim skin.

1.0 INTRODUCTION

Sexual assault is an important health and welfare issue in Australia, with 1 in 5 women and 1 in 20 men from the age of 15, being sexually assaulted and/or threatened (1). Women were found to be most frequently sexually assaulted by friends or acquaintances, followed by intimate partners than strangers, respectively (2). Sexual assault is defined as any non-consensual sexual behaviour and/or acts against a person, in any location, regardless of their relationship to one another (1, 3) It is without saying that sexually-based criminal offences are considered inhumanely abhorrent with the analysis of biological evidence often required for justice to the abused (4). During the assault, minute quantities of DNA, often referred to as trace DNA, can be transferred between a perpetrator, victim and/or scene. Typically, a crime scene is any place where a criminal offence was committed but can include clothing worn by a victim at the time of offence along with the victims' body itself (4). In cases of sexual assault, the body of the victim is the most important, and sometimes only, crime scene available. The amount of time to collect DNA evidence from a victim's body before it is no longer recoverable is still relatively limited (5). Trace DNA evidence can persist on a victim's body or clothing hours after the assault has occurred in qualities sufficient for offender identification (6-9). Biological evidence sampled from the victim's skin can assist with the identification of an assailant and elimination of suspects (6), place an offender at the scene (7), and/or provide corroborative evidence that physical contact has occurred (4, 6). As research has found the assailant is often known by the assaulted, DNA evidence allows, both independently and objectively, for a connection to be drawn between offender, victim and scene (10). Thereby, it is imperative that trace DNA evidence, in alleged sexual assault cases, is correctly collected and examined.

Recovery and collection of offenders trace DNA evidence from a victim is firstly dependent upon the selection of an appropriate sampling method (11). It is vital to collect solely the offender's DNA from the victim, therefore it is vital to secure, aseptically, as much of the perpetrator's cellular material as possible (7, 8). Predominately obtaining the offender's epithelial cells and subsequently DNA (and as little of victim DNA), is critical in the sampling method employed to recover DNA from victim skin (8). Currently, there are several methodologies for collecting offender DNA from the victim's skin, post physical contact including various methods of swabbing and tape-lifting. The efficiency between these sampling techniques for retrieval of offender's DNA from victim skin is yet to be investigated, however, the common standard protocol is to use the double-swapping method. Contrary to recent studies, double-swabbing has been shown to recover more saliva from human skin in comparison to single swab technique and therefore is likely to yield more DNA needed for a conclusive DNA profile (12).

2.0 DISCUSSION

2.1 Recovery of DNA from skin-to-skin contact

DNA profiles are representative of an individual's genome, traditionally presented in the form of an electropherogram (13). When more than one individual contributes to a profile, it is considered a mixed DNA profile. In criminal cases such as sexual assault, where the skin of the victim is sampled for offender DNA, mixed profiles are common. Mixed profiles may be challenging to interpret as individuals can contribute differing quantities of DNA (14). Owing to DNA being a cellular-borne material, it is often transferred directly or indirectly from one surface to another (15). A primary or direct transfer occurs prior, during or post-criminal offence usually through physical contact (16). Alternatively, indirect transfer (secondary transfer) occurs through an intermediate handler that collects DNA from a surface and deposits it to another (15, 16). It is generally the result of everyday interactions and activities (17). For example, hands readily pick up non-self-DNA from one surface, and simultaneously deposit the non-self-DNA with their own DNA, onto another surface (16). As a result, DNA can be transferred multiple times between surfaces and individuals (16) and therefore can cause complications when trying to obtain a conclusive DNA profile (7). There are several core factors known to influence the transfer of DNA onto skin, including the manner of contact causing transfer, shedder status, and the nature of the biological material involved (18). For this reason, the success of a DNA profile is still heavily reliant upon the recovery of the target DNA (offender) and to a lesser extent the background DNA (victim/secondary transfer DNA) (19).

2.1.1 Background DNA

DNA profiles can be readily produced when DNA is available, however, achieving a conclusive DNA profile generally requires the greater abundance of one source of DNA. Trace DNA is responsible for generating the majority of DNA profiles, often resulting from skin cells left on a surface via touch (20), the most dominant DNA profile in a mixture does not always come from the individual who last touched a surface. Electropherogram peaks from the victim may be visible in a DNA profile as well as potential contamination of DNA, present on the skin of a victim and/or offender (8). Owing to self-DNA transferring at a similar rate to any secondary non-self-DNA often referred to as background DNA (7, 17), it is not always possible to distinguish between DNA the offender transfers directly and any background DNA. The complexity of a DNA profile decreases as the ratio of offender DNA to non-offender DNA increases (8). Additionally, lifestyle habits, living circumstances and shedder status can impact on the amount of background DNA present on an individuals' skin. A study by Graham and Rutty reported that those living with partners or family had higher levels of background DNA. This could be problematic in cases of sexual assault, as the offender is frequently known to the victim. An Australian study by Zilkens et al. (2) of 1163 women reported 32.3% of the time the assailant was a friend or acquaintance of the victim and 17.5% of the time, they were intimate partners. In cases of sexual assault, transfer of DNA often occurs through strong physical skin to skin contact or other oral acts resulting in saliva being deposited on the victims' skin. It is crucial when dealing with trace DNA from skin cells, to understand and acknowledge the effects of background DNA on profile interpretation. Therefore, when analysing a mixed DNA profile, it is vital that every allele observed is scrutinised and accounted for (17). While background DNA is

unavoidable, reducing its quantity through the sampling method could be extremely beneficial to DNA profiling.

2.1.2 Shedder status

The quantity of DNA an individual deposit onto a surface in the first instance impacts the dominance of the said individual in a mixed DNA profile. The ability to categorise an individual based on the amount of DNA they deposit via touch is referred to as shedder status and is an area of research under constant debate (21-25) as seen in Table 1. It is hoped that the ability to categorise an individual as a good or bad shedder will help investigators to consider how likely it is that a person's DNA profile would be found on an item (21, 23). A good shedder is defined as an individual who leaves behind enough DNA on a surface, after touching it, to produce a full DNA profile, with a poor or bad shedder only leaving behind a partial DNA profile (24). Given they are a good shedder, it is possible their DNA on the object is the result of indirect, or secondary transfer. An individual's DNA profile can be obtained from an item they had no direct contact with, while the profile of the individual who did touch the item is not detected (24). A study by Goray et al. (21) found good shedders deposited self-DNA at a higher quantity to non-self-DNA. Additionally, occasionally when an individual had a shedding status of poor, non-self-DNA was observed as the major contributor in the mixed DNA profile produced. Szkuta et al. (25) paired good and bad shedders together and had them shake hands before immediately placing their hand on a surface. Poor shedders were unable to deposit enough DNA to obtain complete profiles from their own handprints and were generally undetectable in the good shedder's profile (25). However, it was acknowledged that this only occurs when contact with an item transpires immediately post interaction between individuals (24).

Nevertheless, this is potentially a serious problem when profiling from touch DNA samples, especially if the offender is a poor shedder. Controversially, Phipps and Petricevic (23) argued that being a good or bad shedder is highly variable, quantities of DNA don't just differ between individuals but between an individual's own hands (dominant or non-dominant).

Table 1. – Summary of current research findings on shedder status as of April 2019

Author and year of publication	Can shedder status be determined?	Does washing hands influence shedder status?	Does shedder status differentiate between an individuals' hands	Does shedder status influence the impact of no-self DNA
Lowe et al. (24) - 2002	Yes	Yes	N/A	N/A
Phipps et al. (23) - 2007	No	Yes	Yes	N/A
Goray et al. (21) - 2016	Yes	No	No	Yes
Fonnelop et al. (22) - 2017	Yes	No	N/A	Yes
Szkuta et al. (25) - 2017	Yes	No	N/A	Yes
N/A = factors that were not investigated or commented on within their study				

Shedder status has been thought to differ between a person's hands as individuals shed a more significant amount of DNA from their dominant hand than their non-dominant (23). A person's dominant hand potentially has a greater quantity of loosened cells compared to their non-dominant hand, generated through an increased amount of use (23). Majority of the research in this area (21, 26, 27), however, does not support this study, with no significant difference found between the amount of DNA deposited by either hand. One possible reason for the differing results of each study may be explained by differences in laboratories (23), including the sensitivity of equipment used and the sampling and extraction methods employed. In addition to hand dominance, Phipps and Petricevc

identified the time since hand washing had a major impact on DNA deposited, thus influencing shedder status categorisation.

A point of consideration throughout literature is whether or not shedder status is a reflection of personal habits, in regard to how often individuals wash their hands. A study by Phipps and Petricevic found individuals who went the longest without washing their hands, generally transferred higher quantities of DNA (23). Therefore, the washing of hands can negatively affect the amount of DNA deposited (23, 24). In contrast to this, a more recent study by Goray et al. (21) found no significant difference between deposits made with and without an individual washing their hands, with participants found to shed at a consistent rate, regardless of time since washing. Multiple studies have produced similar results, concluding washing hands to have no influence on the amount of DNA shed (22, 25). However, time since washing hands in this study was not standardised, only roughly estimated. While no reportable significant difference was observed, the majority of samples taken 15 minutes after washing hands ranked amongst the lowest made by the relevant participant (21). No exact relationship between time of hand washing and the amount of DNA shed is observed thus far (24). Additionally, Bright et al. (28) reported that individuals with relatively drier hands present as better shedders. Loss of moisture can cause dry skin to flake and chap, increasing the number of cells shed, resulting in an individual being categorised as a good shedder (28). Shedder status may not be overwhelmingly influenced by a single washing before depositing, as their hands might be dry as a result of lifestyle and daily activities. An individual could potentially alter their shedder status by changing their hygiene habits or use of their hands. This highlights the difficulty in classifying a persons' shedder status as either good or bad indefinitely. Understandable, shedder status has been an area of constant disagreement with a

consensus yet to be reached on whether an individual can be conclusively categorised as a good or bad shedder. Table 1 presents a summary of the current literature and their conclusions on the various concerns with shedder status. Recent studies are generally more supportive of the idea that some individuals consistently deposit more DNA than others (21, 22, 25, 27). Shedder status needs to be considered and is of great importance when analysing trace DNA evidence deposited by hands.

2.2 Recovery of DNA from Saliva

In criminal acts, such as sexual assault, physical skin to skin contact is not the only way offender cellular material can be deposited onto a victim's skin. The perpetrator's saliva may be transferred through actions such as licking, kissing, biting, sucking or spitting (4-6, 29). Saliva itself does not contain DNA, but it is present within the cellular material that is sloughed naturally with the liquid, from the mouths inner lining (29). Epithelial cells and glandular cells from the offender's mouth are therefore transferred to a victims' skin (4, 29). As wet biological material transfers more readily then dried, therefore, DNA recovered from small volumes of liquid cellular material is higher than that recovered from touched objects (30). While blood is the preferred source of DNA for the generation of a genetic profile, saliva can yield high quantities of DNA with minimal to no degradation (5). Additionally, a study by Kenna et al. (29) found saliva can persist on skin for at least 96 hours, with a DNA profile matching the donor successfully generated at this time. However, as with skin-to-skin contact DNA, hygiene activity has been identified as potentially influencing the recovery of DNA.

While it may be instinctive, victims of sexual assault are encouraged to refrain from showering as the predominant belief is that any form of washing will remove salivary

evidence from a victims skin (5). A study by Williams et al. (5), however, concluded that even after showering, salivary DNA remains on the victim's skin and male DNA can be successfully recovered at a rate of 60%. While this is promising, as with any form of trace DNA, the biggest hurdle for the recovery of saliva DNA is identifying where on the body is was deposited. The nature and extent of the interaction between offender and victim may leave a visible injury, however, the majority of saliva transfers leave no visible trace (5). When no apparent injury is present, the examiner is reliant on victims statement relaying the offender's actions during the assault to help them locate the areas on the victims' body where saliva was deposited (5). Unfortunately, victims can be unclear when recalling details of the assault against them, commonly due to alcohol and/or drug consumption at the time (6). Presumptive testing for amylase can help locate the potential presence of saliva before sampling (6). When sampling sexually assaulted women, it is important to avoid causing any secondary victimisation by performing unnecessary sampling (10). When choosing a sampling method, it is important to consider the potential of secondary victimisation, as well as the practicality of the technique and cost.

2.3 Swabs as a sampling method

For many years, swabs have been used within the field of forensics, for the collection of trace DNA evidence from a wide range of surface types (31). Swabs act as intermediating devices that collect and retain DNA until processing and analysis commence (32). To obtain a reportable genetic profile, it is imperative that the collection of biological material is performed correctly (10). There are, however, discrepancies regarding the correct application technique of a swab across the target surface. While it is impossible to ensure every examiner applies the same amount of force and motion, there are some universally

agreed upon guidelines for swabbing. Generally, if the target surface is dry, such as skin, the swab should be moistened first (4), as dry swabs are unable to solubilise dried biological material resulting in inefficient sampling (31). Additionally, when collecting biological material from skin, the swab should be rotated, ensuring every part of the bud comes into contact with the target surface (5, 33, 34). Traditionally, a single moistened cotton swab is used for the recovery of biological material (10, 29). However, there are many types of swabs of different design, size and shape that are available and need to be carefully selected dependent upon the purpose of their use (4).

2.3.1 Swab types

The effectiveness of a swab can depend on a range of factors, arguably the most important properties of a swab are its composition and design (35). Swabs fall into one of three categories, wound swabs, flocked swabs or pad swabs (35, 36). Cotton or rayon swabs are most commonly used and fall into the wound category, referring to the way many fibres, or one single long fibre, is tightly wound around a shaft to make a bud (35-37). Research suggests due to its tightly wound structure these swabs have a limited surface area for the collection of biological material (31). Alternatively, flocked swabs are made from short nylon strands that have been glued onto a shaft, protruding directly outwards, increasing the surface area available for collection (31, 35, 36). Lastly, foam swabs fall into the third category of pad swabs, they contain a sleeve of foam that is attached to the end of the swab shaft (35, 36). It is suggested that the foam matrix gives the swab a more flexible nature, allowing for better penetration into substrates, resulting in a greater sample uptake (35). It is unclear as to which swab type is superior, as results vary depending on the substrate and type of biological material being collected.

Often the choice of swab type used for the collection of trace DNA is made based on convenience and price (34). A study by Verdon et al. (35) found some types of swabs are significantly more effective than others for sampling various forms of biological material from different surface types. For example, foam swabs outperformed all other swabs for the collection of all biological material from wood, while flocked swabs failed to yield the greatest quantity of DNA for any substrate or biological material tested (35). Unfortunately, skin as a target surface was not investigated and increased efficiency of sampling did not always correspond to an increased extraction efficiency (35). Sample collection methods need to be able to release DNA just as effectively as they collect the material during extraction. Manufacturers of the flocked nylon swab claim its design allows for increased absorption and greater release rate (37). Results from an investigation by Gilberto et al. (38) found flocked swab had an instantaneous release rate of more the 80% of absorbed material. Contradicting this, a more recent study by Brownlow et al. (37) reported flocked swabs were not superior to cotton swabs, with both capable of retrieving and releasing high percentages of DNA. However, there is no guarantee that the use of one swab will collect the entirety of biological material on a surface (10). It is now common practice for the double swabbing method to be performed (34), as first advocated by Sweet et al.

2.3.2 Double swabbing

The double swabbing method involves the application of a single moistened swab to the area of interest, followed by a single dry swab (8, 10, 29, 33). Succeeding the collection process, the cellular material from both swabs then undergoes joint extraction (10, 33), ensuring the greatest quantity of DNA is obtained. In theory, the use of two swabs ensures that any cells loosened but not collected by the first swab, are secured by the second (8).

Recently, studies have questioned whether or not the second swab is able to recover any DNA after the first. Pang and Cheung (33) addressed this problem in an experiment by performing the double swab method but extracting the swabs separately. The second swab was able to recover enough cellular material to produce a DNA profile (33). It is reasonable to assume the two-swab method has the potential to double the quantity of DNA recovered, by pooling the cellular material collected by both swabs. In the study, however, trace DNA was recovered from high trafficked target surfaces such as light switches and door handles and not skin. The initial advocation for the double swab method came from Sweet et al. (12), for the recovery of saliva from skin. Skin surfaces used, however, where on cadavers, allowing for a much more controlled environment, potentially reducing the influence of background DNA or external influences, such as clothing. This may explain why several recent studies focusing on the recovery of DNA from the skin of living participants reported contradicting results.

Ferreira-Silva et al. (10) investigated the recovery of DNA from semen on skin, comparing the double swabbing method to the use of a single swab. It is argued that if the first swab absorbed all moisture deposited on the skin, then the dry swab may not collect any cellular material from the sample site (6). If this is true, a single swab would be able to recover similar quantities of DNA as the double swab. Research by Ferreira-Silva et al. (10) reported no statistically significant difference between the quantity of DNA or quality of genetic profiles by either technique. These opposing results suggest the second swab is unnecessary and unable to collect enough DNA from skin to have an impact on the resulting profile. Given the lack of a difference between the single and double swab method, Ferreira-Silva et al. (10) propose that the use of two swabs holds no financial or practical benefit. While numerous studies utilise the double swabbing technique for the recovery of

trace DNA from various surfaces (23, 33, 39), very few have compared it to other methods available.

An investigation by de Bruin et al. (8) compared the double swabbing method to a form of tape-lifting known as stubbing. When it came to securing offender DNA, both techniques performed similarly, with the DNA profiles generated containing an equal number of detected alleles and peak heights (8). Small difference regarding the amount of victim DNA recovered where reported, with less victim DNA collected by the double swabbing method, producing a better offender to victim ratio (8). In cases of sexual assault, the offender's cellular material is collected from a victims' skin, therefore their DNA is unavoidable. Nevertheless, to minimise the disruptive influence of the victim skin in a DNA profile, the ratio of offender DNA to victim DNA should be as high as possible (8). This may be due to the difference in contact from a swab compared to tape. Little force is exerted, ad contact between swab and skin is minimal, reducing the level of victim DNA collected and detected (8). Further research is required to confirm this theory, especially considering that the force an individual applies to a swab can vary between sample collectors. Numerous studies utilise the double swabbing method from various surfaces (23, 33, 39), however, the superiority of this method over others is yet to be universally agreed upon.

2.5 Tape-lift as a sampling method

Tape-lifting is another well-established sampling method, previously applied for the collection of loose microscopic materials, such as hairs and fibres (40). Today, adhesive tapes can be used for the collection of minute quantities of cellular material from a range of forensic exhibits, particularly textiles (41, 42). This method gained increased popularity and momentum as a sampling technique, due to its ability to sample from large surface

areas (43) and collect evidence without damaging the substrate (40). Furthermore, tape has been found to be better suited for the recovery of epithelial cells from porous substrates (44) and inappropriate as a sampling method from wet surfaces (45). Due to the adhesive nature of tape, unlike other collection methods such as swabbing, no additional moisturising agent is required. There is no time needed for drying, and no complications with potential water-soluble contaminates (29).

Cellular material is transferred from the substrate to the tape by continuously pressing the adhesive side across the target surface (29), collecting and detaining the DNA within the glue of the tape. The tape-lifting method is generally considered to be quick and easy to perform, as well as cost-effective (28). The traditional process of tape-lifting is usually carried out by placing the tape around the gloved fingers of the examiner (8). However, researchers believe this could be a cause of contamination. To increase the distance between the target surface and the examiner and decrease the risk of contamination, the stubbing method was created as another alternative form of tape-lifting (8, 45). Instead of wrapping tape around gloved fingers, adhesive double-sided tape is mounted onto a scanning electron microscopy stub (8, 45). The methodology is the same however, with the adhesive being continuously pressed onto a surface until the tape is saturated, no longer showing any adhesive properties (8), before DNA extraction is performed. Direct extraction of tapes is favoured over swabbing the tapes adhesive for collected material, as this was found to be tedious and resulted in an incomplete retrieval of DNA (42). However, extraction of DNA straight off tape may be challenging due to the stickiness of the tapes adhesive (46) and its more complex chemical nature (42). Barash et al. (47) compared a range of adhesive tapes and concluded that while there are many tapes suitable for the collection of biological material, not all of these tapes are suitable for extraction.

Unfortunately, reliable information on the type of DNA extraction method that should be used for specific adhesive tapes is scarce (42).

Majority of research thus far has investigated tape-lifting as a recovery technique for cellular material from textiles, and not from skin. One study by Albujja et al. (48) advocated for the use of tape-lifts as an alternative, and less invasive method for collecting reference biological evidence for DNA analysis. Two types of traditional adhesive tapes and a single swab were compared to determine which technique recovered the highest concentration of DNA from epithelial cells (48). Their results found, however, that neither method was superior, with the traditional tape lifting method found to yield similar quantities to the single swab (48). This supports the notion that DNA can be recovered from skin using the tape-lifting method, however, an investigation by de Bruin et al. (49) focussed more specifically on securing offender DNA from victim skin. The authors compared the tape-lifting method known as stubbing to the double swab technique. While the stubbing method recovered a higher concentration of DNA, this included more victim DNA (8). In order to obtain a useful DNA profile, the ideal sample contains as little background DNA (victim), and as much target DNA (offender) as possible (45). Swabs applied with little force have minimal contact with skin, whereas tapes are dabbed onto a surface continuously until no longer adhesive. An increased amount of victim DNA could be due to the more intense contact that occurs between adhesive tape and skin (8). Nevertheless, adhesive tapes when saturated were found to carry more cellular material then swabs, therefore, the offender DNA may be the limiting factor (8). Had there been more offender epithelial cells on the victim skin, the tape lift may have recovered a higher ratio of offender DNA to victim DNA. With sexually related crimes, the quantity of DNA is generally at minute levels, this may work against the use of traditional tape-lifts as an ideal sample collection method.

The use of mini-tapes may prove more promising, however, as they are smaller and are pressed not dabbed onto a surface.

2.5.1 Mini-tape

A new tape-lifting method, referred to as mini-tapes, has proven to be reliable and easy for the sampling of DNA from textiles (11, 50). Similar to traditional tape-lifting methods, mini-tapes are double-sided adhesive strips that are repeatedly pressed across a surface in order to transfer cellular material from the substrate to tape (29). This technique is commonly applied by forensic practitioners in laboratories and at crime scenes to collect and preserve evidence (11). As previously stated, the superiority of adhesive tape to recover epithelial cells from textiles has been confirmed, the use of adhesives on skin is still a very under researched area. A study by Kenna et al. (29) is one of the only available research that has compared the mini-tape method to other sampling methods for the recovery of DNA from skin. Their research found that when recovering salivary DNA from skin, the mini-tape method was generally more effective then swabbing (29). They also noted the use of adhesives is a lot faster, due to a lack of drying time. The mini-tape method could potentially be an improved sample collection method to recover offender DNA from victim skin, unfortunately, no such comparison of the mini-tape has been investigated to date. Regrettably, there is no one study comparing all the available sampling methods and even an accumulation of all the current research, as seen in Table 2, gives inconclusive and sometimes contradictory results. In general, older studies report significant differences between methods, whereas more recent studies have found little variation. There is a need for a collection method that is fast and simple like a tape-lift but can yield a greater offender to victim ratio, as a swab.

Table 2 – A summary of the studies comparing sampling methods for the recovery of offender DNA from victim skin.

	Sampling Methods Compared			
Author and Date of Publication	Double Swab	Single Swab	Tape-Lifting/ Stubbing	Mini-Tape
Sweet et al. (12) - 1997				
Kenna et al. (29) -2011				
de Bruin et al. (8) - 2012				
Albujja et al. (48) - 2018				
Ferreira-Silva et al. (10) - 2019				

KEY	
	A green tile indicates this method was found to be more effective
	A red tile indicates this method was found to be less effective
	Yellow indicated no significant difference was found between the sampling methods
	Tile left blank to indicate the sampling method was not compared within the study

2.4 Alcohol wipes/solution

When collecting cellular material from evidentiary samples by swabbing, it is common practice for forensic laboratories to use sterile water as a moistening agent (19). Some studies have compared water to detergent-based swabbing solutions, with the later consistently found to outperform water (19, 51). Despite the lack of research confirming water as a superior solution for retrieving DNA, it is still the most commonly used solution. Another alternative considered more recently is the use of alcohol swabbing solutions, particularly ethanol as a moisturising agent. Ethanol is easily accessible and a weak enough acid that it can be applied to most surfaces (52). Although it is very mild, it could be uncomfortable on skin if there are little cuts or grazes presents, this is an issue that is dependent on the person and will most likely need to be accessed on a case by case basis. Within the field of forensics, ethanol is mostly employed as a fixative to preserve post

mortem tissue specimens, as it has been found to decrease DNA degradation (53), due to its natural antibacterial properties (48, 52). The use of 70% alcohol as a moistening agent has the ability to delay, even prevent, microbial growth for up to 5 days in less than ideal storage conditions (52). Standard cotton swabs moistened with sterile water need to be dried before they can be stored to stop bacterial degradation of DNA. As a swabbing moistening agent, it has the added benefit of a short drying time as the reagent evaporates quickly (52). This reduces the time needed to allow for the swab to dry before storing it appropriately. Despite the potential benefits, alcohol is rarely employed as a swabbing solution and there is very little research investigating its ability to recover epithelial cells from any surface, let alone skin. The closest pre-existing method is the self-wetting foam swab, referred to as a mini-popule.

The swab handle of a mini-popule has an ampoule containing 91% isopropyl alcohol (IPA) (35). Unfortunately, its employment as a sample collection method compared to other swab types is not well published, except in a publication be Verdon et al. (35). The authors reported less than favourable results with the self-wetting foam swab performing poorly, consistently ranking lowest in efficiency when recovering biological material from a range of surface types (35). It was hypothesised that due to the lower polarity of IPA then water, the interaction between the solution and cellular material was less efficient than other water-based solutions (35). Nevertheless, while these results aren't ideal, alcohol wipes containing 70% isopropyl have many potential benefits and could be employed as a collection method. Not only are alcohol wipes easily accessible and inexpensive, but they are also pre-moistened. This allows for a simple and easy sampling procedure, perfect for early evidence kits as no additional solution is required, and the wipe can be stored without the wait of drying. Early evidence kits are recommended to not only preserve forensic

evidence, but as a comfort to the victim when there is a delay before they are able to access forensic medical services (54). Verdon et al.'s (35) study reported the mini-popule mechanism did not consistently work, failing on multiple occasions (35). The use of a wipe instead of a self-wetting swab could prevent such a problem. Given the inconsistency and lack of comparison of all current collection methods, there is no good reason why the use of alcohol wipe as a new technique shouldn't be explored.

3.0 EXPERIMENTAL DESIGN

3.1 Sample collection

Two independent pairs will be sampled for two mock scene scenarios, consisting of one male 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'. The pre-determined volume of buccal rinse ('lick') from the offender will be then be pipetted onto the neck of the victim and allowed to dry. 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

3.2 DNA extraction and quantification

All DNA from samples will be extracted using the Qiagen extraction method and quantified using a Quantifiler Trio.

3.3 Data analysis

The data output from the Quantifiler Trio will be analysed to determine the total DNA concentration and offender DNA concentration (Y-DNA) yielded from each sample. This information will then be used to produce a ratio of male to female DNA for comparison. Average total male DNA yielded for each sampling method will be calculated and compared for each mock assault scenario. An appropriate 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 the most effective and efficient sampling technique for the recovery of an offender's DNA after skin-to-skin or saliva-to-skin contact has occurred. Several pre-existing sampling methods, including; the double swab, single foam swab, mini-tape and scotch tape-lift method will be analysed, for the recovery of offender cellular material from victim skin. In addition, alcohol wipes as a potential recovery method will also be assessed. This study aims to determine an optimal sampling method for the recovery of cellular material from victim skin.

1. Determine if the use of alcohol wipes is effective for the recovery of male (offender) cellular material from female (victim) skin.
2. Evaluate which sampling method yielded the greatest concentration of male (offender) DNA, given physical skin to skin interaction.
3. Evaluate which sampling method yielded the greatest concentration of male (offender) DNA, given offender saliva to victim skin interaction.

4.1 Experimental hypothesis 1:

H_1: After physical contact, whereby male cellular material is deposited onto a female's skin, alcohol wipes will successfully recover cellular material, resulting in male DNA of the donor being detected by the Quantifiler®Trio.

4.2 Experimental hypothesis 2:

H_1: After a simulated mock assault, whereby the wrist of a female victim was grabbed by a male offender, the double-swab method will yield the greatest amount of offender DNA

from the victim skin when compared to the mini-lift, scotch tape-lift, foam swab and alcohol wipe sampling methods.

4.3 Experimental hypothesis 3:

H_1: The greatest yield of offender DNA recovered from a victim's skin after a simulated mock assault, whereby a pre-determined volume of buccal rinse from a male offender was pipetted onto the neck of a female victim, was achieved using the double swab sampling method compared to alternative methods using a mini-lift, scotch tape, foam swab and alcohol wipes.

5.0 CONCLUSION

In cases of sexual assault, the body of the victim is often the most important and only available crime scene DNA evidence can be collected from. The production of a successful DNA profile strongly relies on the recovery of target DNA from their skin in the first instance. This paper identified several factors that could potentially influence or hinder this retrieval process, including background DNA, shedder status and washing. Despite the contradicting results of many studies in this field, it is largely agreed that the ideal collection method needs to be able to recover the highest quantity of offender DNA and as little victim DNA as possible. For this reason, of the methods discussed, swabbing could be considered a better technique for recovering DNA from skin, compared to tape-lifting. However, the supposed superiority of the double swabbing method is underwhelming with little research confirming this technique is significantly better than other methods, particularly when compared to the simple use of a single swab. Unfortunately, an accumulation of all the current research offers inconclusive and sometimes contradictory results regarding which

sampling process is the most beneficial. Given the lack of overwhelming success by any method within the research so far, the introduction of a new technique should be explored. Alcohol wipes are self-moistened, cheap and easily accessible, their potential to recover cellular material from skin needs to be explored further. There is a need for a single study to compare all the available and potential collection methods specifically for the recovery of offender DNA from victim skin, as it is important to continue identifying and upholding best practice for forensic sampling.

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- Part Two -

MANUSCRIPT

SAMPLING METHODS FOR THE RECOVERY OF OFFENDER CELLULAR MATERIAL FROM VICTIM SKIN SURFACES

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ABSTRACT

Sexual assault is an extremely serious criminal offence with major health and welfare repercussions. Minute quantities of DNA can be transferred between the offender and victim as a result of skin-to-skin or saliva-to-skin contact. The body of the victim is sometimes the only crime scene available to collect DNA evidence, therefore it is imperative that any trace DNA present is correctly collected. Previous research on sample collection methods have seldom focused on human skin as a target surface, and to date, there has been no single comparative study assessing all available techniques. Within this experiment, five sampling methods were compared and analysed, including alcohol wipes proposed as a new recovery method, along with pre-existing techniques known as the double swab (wet/dry), single foam swab, scotch tape-lift and mini-tape. Two different scenarios were investigated to simulate events of a sexual assault, resulting in saliva to skin or skin to skin contact between offender and victim

The double swabbing method is currently considered the method of choice for the recovery of offender cellular material from victims' skin surface. Of the five different sampling methods investigated, the mini-tape technique secured the highest concentration of offender DNA (0.269 ± 0.301 ng/µl). while the double swabbing method yielded the lowest concentration (0.045 ± 0.017 ng/µl) for the recovery of saliva from human skin. While the sample size will need to be increased in future experiments the promising results demonstrated from the mini-tape should be considered by forensic medical services for the sampling of sexually assaulted victims under these circumstances. With these findings, it is recommended that the employment of the double swabbing method is re-evaluated. This study was the first to utilise alcohol wipes for the recovery of DNA from skin and found

this method yielded the second highest concentration of offender DNA securing 0.15 ± 0.105 ng/µl. Further research is recommended before implementation, nevertheless, the alcohol wipes would be a perfect inclusion for early evidence kits, or as any immediate alternative, due to being relatively ubiquitous to health campuses and clinics. Unfortunately, the results of this study were not statistically significant due to a low sample size (n = 4), it is recommended more samples should be taken in future studies to overcome this limitation.

Key Words*:* Trace DNA; Sampling Methods; Mini-Tape; Double Swab; Saliva on Skin

1.0 INTRODUCTION

Sexual assault is defined as any non-consensual sexual act and/or behaviour against a person, in any setting, regardless of their relationship to each other (1, 3). Sexual assault is considered a major health and welfare issue in Australia, as, from the age of 15, one in five women and one in 20 men are sexually assaulted and/or threatened (1). Minute quantities of DNA, often referred to as trace DNA, can be transferred between a victim, perpetrator and/or crime scene during an assault. Commonly, any place where a criminal offence is committed is considered a crime scene, including the body of the victim themselves (4). In sexual assault cases, the body of the victim is the most important, and sometimes the only, existing crime scene. Biological evidence sampled from the victim's skin can place the assailant at a scene (7), offer corroborative evidence that physical contact has occurred (4, 6), and ultimately assist with the identification and elimination of suspects (6). Understandably, it is imperative in alleged sexual assault cases for trace DNA evidence to be correctly collected and examined as soon as possible. The successful collection and recovery of trace DNA evidence belonging to the offender from a victim's body is firstly dependent upon the selection of an appropriate sampling method (11). Currently, several methodologies are available for the collection of cellular material from various substrates, including several swabbing and tape-lifting techniques.

Within the field of forensics, swabs have been used for the collection of trace DNA evidence from a wide range of substrate types for many years (31). There are many different types of swabs, ranging in design, size and shape available dependent on their intended use (4). Cotton swabs fall into the first category known as wound swabs, referring to the way one single long fibre, or many fibres, are tightly wound around a shaft forming a bud (35-37).

Due to the tightly wound structure of these swabs, the surface are available to collect biological material may be limited (31). Alternatively, flocked swabs are thought to have a larger surface area for collection as they consist of short nylon strands glued to a shaft, protruding directly outwards (31, 35, 36). Foam swabs fall into the third and final category of pad swabs, consisting of a sleeve of foam attached to the end of a swab shaft (35, 36). Unfortunately, skin as a target surface has yet to be investigated to determine which swab type is superior for the collection of cellular material. However, a single moistened cotton swab is traditionally used for the recovery of biological material (10, 29).

While it is impossible to ensure every individual examiner uses the same swab type and applies the same amount of force when swabbing, there are some universally agreed upon guidelines. If the target surface is dry, such as skin, generally the swab should be moistened first (4), as dry swabs cannot solubilise dried biological material, resulting in ineffective sampling (31). Furthermore, swabs should be rotated to ensure every part of the bud comes into contact with the target surface (5, 33, 34). Nevertheless, it is not guaranteed the use of one swab can collect all available biological material on a surface (10). Therefore, it is now common practice to perform the double swabbing method (34), as first advocated by Sweet et al.

The double swabbing method involves the initial application of a single moistened cotton swab to the area of interest, followed by a single dry swab (8, 10, 29, 33). Succeeding the collection process, both swabs then undergo joint extraction (10, 33). In theory, any cellular material loosened by the first swab, but not collected, is then secured by the second (8). However, it is argued that should the first wet swab absorbs all the moisture deposited on the skins surface, then the dry swab would not collect any cellular material (6). Pang and

Cheung (33) addressed this problem and found the second swab alone was able to recover enough DNA to produce a successful DNA profile. It is reasonable to expect the double swabbing method could, therefore, recover double the amount of DNA than a single swab. Contradicting this, a study by Ferreira-Silva et al. (10) comparing the double swabbing method to the use of a single swab and reported no statistically significant difference between the quantity of DNA recovered by either technique. While there are several studies utilising the double swabbing method, the superiority of this technique over others is yet to be universally validated.

Another well-established sampling method is the use of adhesive tapes, originally employed for the collection of loose microscopic material, such as hairs and fibres (40). Today, the tape-lifting technique is used to collect trace DNA from a range of forensic exhibits, particularly textiles (41, 42). The traditional tape-lifting process is usually carried out by wrapping tape around an examiner's gloved fingers, with the adhesive side facing outwards (8). The tape is then continuously dabbed across the target surface until saturated (29). Unlike other sampling methods such as swabs, due to the adhesive nature of tape, no additional moisturising agent is required. Therefore, there are no complications with water-soluble contaminants and no time needed for drying (29). An investigation by de Bruin at al. (8) compared the use of an adhesive tape to the double swabbing method, for the recovery of offender cellular material from victim skin. Both methods performed equally well when it came to securing offender DNA, however small differences were reported regarding the quantity of victim DNA recovered (8). While the victims DNA is unavoidable when sampling from their own skin, to minimise the disruptive influence of their DNA when profiling, the ratio of offender DNA to victim DNA should be as high as possible (8). The increased amount of victim DNA recovered by tape may be due to the

difference in contact between the sampling device and skin. When little force is exerted, the contact between a swab and skin is minimal, therefore an increased amount of victim DNA when tape-lifting, could be due to the more intensified contact that occurs between adhesive and skin (8). The use of mini-tapes may prove promising, as they are pressed onto the skin rather than dabbed, however this could potentially recover more victim DNA.

Mini-tapes are one-sided adhesive strips, proven to be reliable for the sampling of DNA from textiles (11, 51). Research by Kenna et al. (29) is the only available study that has investigated the use of the mini-tape to recover cellular material from skin. Their study found the mini-tape method was generally more effective then swabbing when recovering saliva from skin (29). Unfortunately, there is no one study comparing all the collection methods available when sampling from skin. Older studies have reported significant differences between methods, whereas more recent studies have found little variation. There is a need for a collection method that is quick and simple to perform such as a tape-lift that can yield a greater offender to victim ratio.

Given the lack of universal support for any single sample collection method, new techniques should be explored. Alcohol wipes are easily accessible to medical and healthcare workers and are inexpensive, sterile and pre-moistened. This allows for a simple and easy sampling process, as no additional solution is required. Wipes can be stored without drying, ideal for early evidence kits. There is a need for a single study comparing all available and potential collection methods, specifically for the recovery of offender DNA from victim skin, as it is important to continue identifying and upholding best practice for forensic sampling.

2.0 MATERIALS AND METHODS

2.1 Mock Assault Design

Two independent pairs were sampled for two mock scene scenarios, consisting of one male playing the role of the 'offender', and one female playing the role of the 'victim'.

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

To determine the volume of saliva to be pipetted onto the victims' neck, a sheet of filter paper was weighed, then licked and weighed again. The weight of the paper was rounded up and converted to microlitres and represents the average amount of liquid that would be deposited by the act of licking. Each offender deposited saliva into a sterile container, the pre-determined volume of 100 µl of saliva was then pipetted onto both sides of the neck of their respective victims. Once the saliva had dried, the victim was then allowed to go about their everyday activities for six hours, avoiding direct contact to the area and instructed not to wash. After a six-hour time period, the neck of the victim was then sampled by one of the sampling methods.

2.1.2 Scenario 2: Detaining of Victim via Wrist Grab

The offender grasped the wrist of the victim with a firm grip, without causing pain, for a 30 second time period. To simulate restraint, the victim rotated their wrist externally and internally 180 degrees. The victim then went about their everyday activities for six hours, avoiding any direct contact with the area and instructed not to wash. After a six-hour time period, the wrist of the victim was then sampled by one of the five sampling methods investigated.

2.1.3 Sampling

Samples were taken from the skin of the victim, from either side of their neck and the forearm area of both arms. The process was performed once every day for five days with a different sampling method employed each day.

2.1.3.1 Double Swabbing

The double swabbing method was performed using two sterile rayon-tipped swabs (Copan). As per the method outlined by Sweet et al. (12), the target area was swabbed using a wet swab first, followed by a dry swab. The swab head was rotated ensuring every side of the swab head came into contact with the target area. Swabs were returned to their protective sterile case, labelled and stored in a fridge at 4°C until extraction.

2.1.3.2 Foam Swabbing

A single foam-tipped collection swab (Epicentre) was firstly moistened with sterile water. The area of interest was then swabbed, rotating the swab head to ensure the entire swab head came into contact with the skin. Swabs were returned to their protective sterile case, labelled and stored in a fridge at 4°C until extraction.

2.1.3.3 Scotch Tape-Lift

A new roll of scotch tape was used, with the first metre of tape discarded to minimise contamination. A length of approximately 20 cm of tape was then wrapped around the end of three gloved fingers, with the adhesive side facing out. One side of the tape was then repeatedly dabbed onto the target surface were contact occurred, until the tape was no longer adhesive. Samples were labelled and stored in individual Petri dishes at 4°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 marked by the Scenesafe Fast™ brand, the mini-tape was removed from the backing paper. The target surface area was sampled by applying downward pressure to the tape and lifting repeatedly, without dabbing, until the tape was no longer adhesive. Samples were then stored in individual petri dishes at 4°C until extraction.

2.1.3.5 Alcohol Wipe

A Primaswab alcohol wipe saturated with 70% v/v Isopropyl Alcohol was removed from packaging and unfolded. One side of the wipe was wiped across the entire target surface multiple times. Samples were then stored in individual petri dishes at 4°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 600 µl, and the total volume of Buffer ATE was 40 µl. After extraction, all samples were labelled and stored in at -20°C.

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 and Trio DNA Quantification user guide in a 384 well with 20 µl reactions. All samples were thawed, vortexed and centrifuged before being added to their respective wells.

2.4 Data Analysis

The Quantifiler®Trio data output allows for the quantitative and qualitative assessment of total human and human male DNA. The data output attained from the small amplicon signal represents total human DNA, while the Y-target amplicon signal represents the total concentration of male (offender) DNA detected for each sample. The average concentration of DNA 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.

2.0 RESULTS AND DISCUSSION

Past research comparing sample collection methods has rarely focused on human skin as a target surface, with no comparative study assessing all available techniques. Five sampling method were compared and analysed in this study including double swab, single foam swab, scotch tape-lift, mini-tape and alcohol wipe techniques. Two different scenarios were investigated to simulate events of a sexual assault, resulting in saliva or skin to skin contact between offender and victim. Reference samples produced by female participants were found to contain no male DNA, and vice versa for male participants. Data from the small and Y-target amplicon signals were used for the comparison of method and were found to have high amplification efficiencies of 91.57% and 97.26%, respectively.

3.1 Scenario 1: Offender DNA Concentration

The optimal sampling method should be able to secure the greatest amount of offender DNA possible when securing offender DNA from the skin of a victim (8). Within this study, the offender was played by a male, therefore, the quantification data attained from the Y-

target amplicon signal represents the total concentration of offender DNA detected for each sample. There was no significant difference between the average concentration of offender (male) DNA recovered by each sampling method, determined by a one-way ANOVA ($F(4,15) = 1.516$, $p = 0.248$). A low number of samples ($n = 4$) may explain why differences observed were not statistically significant, more samples should be taken in future studies to overcome this limitation.

Of the five different sampling methods investigated, the mini-tape technique secured the highest yield of DNA (0.269 ± 0.301 ng/µl), with alcohol wipes yielding the second highest concentration (0.15 ± 0.105 ng/µl) (Figure 1). A study by Kenna et al. (29) reported similar results, with the mini-tape performing consistently better then swabbing when recovering saliva from skin. While the sample size will need to be increased in future experiments the promising results from the mini-tape should be considered by forensic medical services for the sampling of sexually assaulted victims, this could be extremely beneficial in successfully identifying an offender by their DNA.

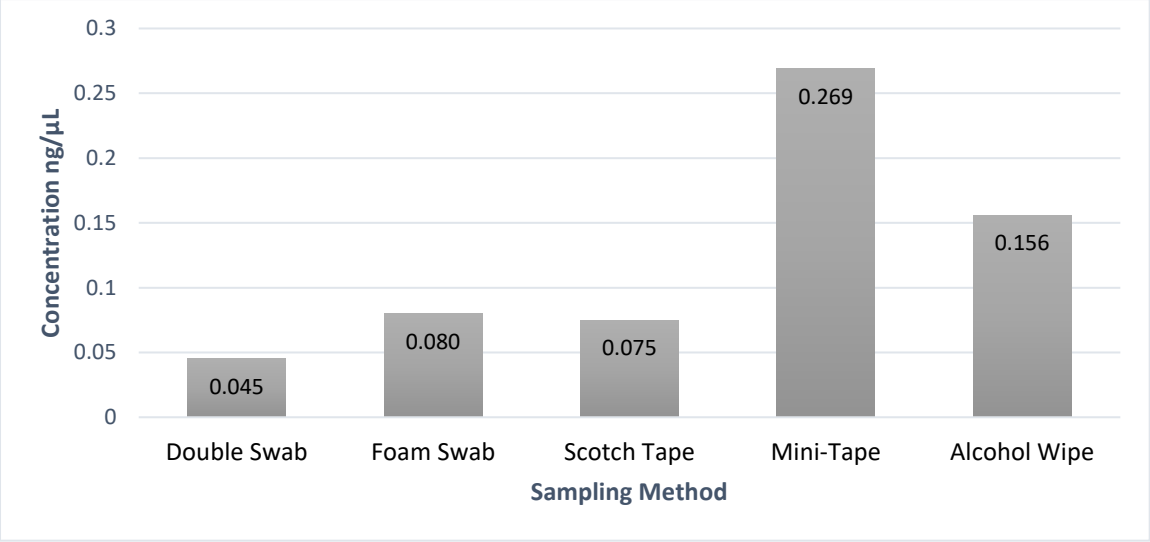

Figure 1. ***Average concentration (ng/µl) of male (offender) DNA recovered for each sampling method after simulated slobber/licking of victim's neck.***

The double swab, foam swab and scotch tape-lift all collected between 0.045 and 0.080 ng/µl of DNA from saliva (Figure 1). The double swabbing method recovered the lowest concentration of offender (male) DNA with a mean concentration of 0.045 ± 0.017 ng/µl. This is less than ideal, given the double swabbing technique is currently the method of choice for the recovery of DNA from sexually assaulted victims ((55) Given the theory behind the double swabbing method, this technique should have recovered twice as much DNA as the single swab. However, the single foam swab method recovered a greater mean concentration of male DNA (0.08 ± 0.089 ng/µl) then the double swab. These results are similar to those found in a study by Ferreira-Silva et al. (10), as there was no statistically significant difference between the use of a single swab compared to the double swab. However, it is important to note that rayon swabs were used in the double swabbing method, whereas foam swabs were used for the single swab.

For many years, swabs have been used for the collection of trace DNA evidence from a wide range of substrate types, within the field of forensics (31). There several types of swabs available, and differences in swab design can influence the quantity of DNA recovered. Swabs need to be able to release DNA during extraction just as effectively as they collect cellular material (37). The tightly wound structure of rayon/cotton swabs may have limited the surface area available to collect cellular material (31) and hindered its release. Alternatively, foam swabs have a more flexible nature, allowing for better penetration of substrates, resulting in a greater uptake of sample (35). Verdon et al. (35) conducted an investigation to determine which swab type was best for the recovery of differing biological material from a range of substrates. Foam swabs consistently ranked the highest when sampling saliva (35), regrettably, human skin was not one of the target surfaces investigated. The lack of DNA recovered by the double swab method could be

explained by the first wet swab absorbing all the moisture deposited onto the skin, therefore, the second swab would not collect any additional cellular material (6). However, if only one cotton swab collected biological evidence, the single foam swab still performed marginally better. Given sexual assault is a major health and welfare problem in Australia, and there is no second chance to resample, further research would be very beneficial to determine which swab type is the most efficient to recover biological evidence from skin. Perhaps the employment of the double swabbing method needs to be re-evaluated with the use of foam swabs instead of rayon or cotton.

The last currently available sampling method that was investigated was the traditional tape-lift. The performance of this method was relatively poor (Figure 1), particularly when compared to the other adhesive tape technique, the mini-tape. Potentially owing to a difference in adhesiveness or variation in the sampling procedure. The traditional tape-lifting process is performed by placing the tape, adhesive side facing outwards, around the gloved fingers of the examiner (8). The methodology of both techniques is similar, with the tapes adhesive continuously pressed across a surface in order to transfer biological evidence from the substrate to the tape (29). The mini-tape is a one-sided adhesive strip, that is pressed and removed from the target surface, using a more controlled motion (lifting repeatedly without dabbing) then the traditional tape-lift process. While the adhesive nature of tape means there is no drying time or additional moisturising agents needed; there is no evidence to suggest that incorporating the traditional tape-lift method would be at all beneficial to the sampling of DNA from sexually assaulted victims.

When collecting biological material from a range of surfaces, the use of swabs and tape-lifts is common practice. To date the use of alcohol wipes containing 70% v/v Isopropyl to

recover DNA from skin, had not been investigated. The methodology was simple and quick, and the results were promising with a mean male DNA concentration of 0.156 ± 0.105 ng/µl. The concentration of offender DNA recovered by the alcohol wipe method compared to the swabbing methods was not statistically significant. However, the alcohol wipe recovered the second greatest concentration of male DNA (Figure 1). In Western Australia, the initial response to a sexual assault complaint is provided by police or emergency services, they direct the collection of specimens through the use of early evidence kits (54). Currently, these kits contain two cotton swabs for the collection of biological evidence from skin surfaces using the double swabbing method. These kits are extremely important, especially in a remote location when access to medical forensic examination services are often delayed (54). Alcohol wipes would be a perfect inclusion for early evidence kits as they are pre-moistened, low-cost, easily accessible to healthcare and medical workers and have now been found to recover high levels of DNA. Of course, further research is recommended before implementation, as this is the first study to investigate the use of alcohol wipes for the recovery of trace biological evidence.

3.2 Scenario 1: Ratio of Offender DNA to Victim

When sampling the skin of a victim, their DNA can hardly be avoided, but, to minimise the disruptive influence of their DNA when profiling, the ratio of offender to victim DNA should be as high as possible (8). The higher the percentage of offender DNA recovered, will provide a greater ratio between the offender and victim DNA. The percentage of DNA recovered belonging to the offender was calculated by dividing male DNA concentration by total DNA concentration (Table 1). Of the five sampling methods investigated, the scotch tape technique produced the highest percentage of offender DNA with a mean of 89%.

Both the double swabbing and mini-tape methods produced similar results, with the offender contributing to 87% and 86% of total DNA recovered, respectively.

Table 1. Average concentrations (ng/µl) of total DNA, male DNA and calculated percentage of DNA that was male for each sampling method for scenario 1: slobber/licking of victim's neck

Sampling Method	Total Concentration of DNA (ng/µl)	Total Concentration of Male DNA (ng/µl)	Percentage of total DNA that was male (%)
Double Swab	0.053 ± 0.021	0.045 ± 0.017	87
Foam Swab	0.101 ± 0.109	0.080 ± 0.089	72
Scotch Tape	0.084 ± 0.069	0.075 ± 0.061	89
Mini-Tape	0.306 ± 0.329	0.269 ± 0.301	86
Alcohol Wipe	0.214 ± 0.178	0.156 ± 0.105	78

A previous study by de Bruin et al. (8) found adhesive tape recovered a higher concentration of DNA then double swabbing, but this included a higher quantity of the victim DNA. Given its adhesive nature, the contact between the tape and skin is thought to be more intense then between swab and skin, therefore, contributing to a higher concentration of victim DNA being recovered. The results of this study suggest otherwise, as both adhesive tape methods recovered very low quantities of victim DNA (Table 1). Essentially all sampling methods recovered a relatively high percentage of offender DNA. Nevertheless, the mini-tape is considered the most effective method, recovering the highest concentration of offender DNA producing a high percentage, when sampling saliva deposited on a victim's skin.

3.3 Scenario 2: Offender DNA Concentration

Only three of the five sampling methods investigated were able to recover male DNA from the female victim's skin after physical contact. The mini-tape, alcohol wipe and foam swab methods were all able to recover 0.001 ng/µl (Table 2). Additionally, double swabbing and

scotch tape recovered the lowest concentrations of male DNA in scenario 1 (Figure 1) and were the two methods unable to recover any male DNA in scenario 2 (Table 2).

Table 2. Average concentrations (ng/µl) of total and male DNA for each sampling method in scenario 2: detaining of victim via wrist grab

Sampling Method	Total Concentration of DNA (ng/µl)	Total Concentration of Male DNA (ng/µl)
Double Swab	0.001 ± 0.001	-
Foam Swab	0.002 ± 0.001	0.001 ± 0.001
Scotch Tape	0.001 ± 0.001	-
Mini-Tape	0.002 ± 0.001	0.001 ± 0.001
Alcohol Wipe	0.005 ± 0.004	0.001 ± 0.001

The lack of DNA recovered by any sampling method in scenario 2, given the high levels of DNA recovered in scenario 1, is most likely due to additional influencing factors in the recovery of DNA from skin surfaces. The manner of contact causing transfer of DNA, 'shedder status' and the nature of the biological material can influence the transfer of DNA onto skin (34). Biological material that is wet transfers more easily than dry biological material (30), therefore, the concentration of DNA recovered from liquid cellular material, such as saliva, would be greater than that recovered from touched objects. Furthermore, the epithelial cells of the offender were transferred from their hand to the victims' wrist, an area most likely to be impacted by everyday activities and the wearing of clothes than the neck area, where saliva was deposited. Additionally, the quantity of DNA deposited via touch can vary between individuals. A good shedder is defined as an individual who, after touching a surface, leaves behind enough DNA to produce a full DNA profile, with a poor shedder leaving behind a partial profile or less (24). Had both male participants been poor shedders, the quantity of DNA deposited for recovery in the first instance would have been minimal. The ability to categorise an individual based on the amount of DNA they deposit via touch is an area of research under constant debate. Realistically, not all offenders will

be good shedders, therefore sampling methods need to be able to recover DNA even when minuet quantities are available.

Given some sampling methods were unable to recover any male DNA, those that did, recovered negligible amounts, the percentage of offender DNA was not calculated. No DNA was detected in the negative controls for three out of the five sampling methods. However, minute quantities of DNA were detected in the mini-tape and foam swab negatives, with concentrations of 0.001 ng/µl and 0.003 ng/µl respectively.

3.0 CONCLUSION

Previous research on sample collection methods have seldom focused on human skin as a target surface, and to date, there has been no single comparative study assessing all available techniques. Within this experiment, five sampling methods were compared and analysed, including alcohol wipes as a new recovery method, along with pre-existing techniques known as the double swab, single swab, scotch tape-lift and mini-tape. Two different scenarios were investigated to simulate events of a sexual assault, resulting in saliva-to-skin or skin-to-skin contact between offender and victim. The sample size will need to be increased in future experiments and the promising results from the mini-tape should be considered by forensic medical services for the sampling of sexually assaulted victims. Particularly when trying to recover DNA from saliva deposited onto skin, as the mini-tape method yielded the highest concentration of offender DNA. In addition to determining the most effective method to recover offender DNA from skin, this study also proposed a new method utilising alcohol wipes containing 70% v/v Isopropyl. The alcohol wipe methodology was simple and quick to perform and yielded the second highest

concentration of offender DNA. Further research is recommended before implementation, nevertheless, alcohol wipes would be a perfect inclusion for early evidence kits. The sexual assault research centre in Western Australia currently uses the double swabbing method for the collection of saliva and skin cells from a potential victims skin (55). In this study, however, the double swabbing method recovered the lowest quantity of offender DNA of all five sampling techniques investigated. With these findings, it is imperative that the employment of the double swabbing method is re-evaluated.

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