Collection techniques of touch DNA deposited on human skin following a strangulation scenario

Alketbi, Salem K [1,2*]

[1]University of Central Lancashire, Preston, UK

[2]General Department of Forensic Science and Criminology, Dubai Police, Dubai, UAE

*Tel: 00447774141205; E-mail: alkitbe.11@hotmail.com

Key Points:

- In scenarios of vicious crimes like assault, sexual offences, or even homicide, trace DNA can be recovered from the skin of the victim.
- This study investigated three collection techniques involving cotton and nylon swabs to examine their efficiency for the collection of trace DNA from the human neck following a strangulation scenario.
- There was a significant difference between the three recovery techniques used to recover touch DNA with a cotton swab ($p < 0.05$) and nylon swab ($p < 0.05$), with more alleles observed when the neck skin was moistened with 100 µL of distilled water using a spray bottle before collection for both swabs.

Abstract: Trace DNA is a significant type of evidence for its ability to be collected from touched items or surfaces at crime scenes to link suspects to their crimes. In cases of violent crimes like assault, sexual offences, or even homicide, often touch DNA is collected from the victim's skin. However, the collection of touch DNA from the victim's skin can be complex because of the mixture of DNA present, as there is likely to be a small quantity of the offender's DNA compared to the victim's DNA. Validating different collection methods or techniques can improve touch DNA sampling, therefore, this study investigated three collection techniques involving cotton and nylon swabs to test their efficiency for the collection of touch DNA from the human neck. There was a significant difference between the three recovery techniques used to recover touch DNA with a cotton swab (CS) ($p < 0.05$) and nylon swab (NS) ($p < 0.05$), with more alleles observed when the neck skin was moistened with 100 µl of distilled water using a spray bottle before collection with both swabs.

Keywords: Forensic science; Trace DNA; Touch DNA; DNA recovery; Cotton swab; Nylon swab; QIAamp DNA investigator kit; Quantifiler™ Human DNA Quantification Kit; GlobalFiler™ PCR Amplification Kit

Introduction

Touch or trace DNA is a crucial form of evidence due to its potential to be collected from any touched items or surfaces, enabling the linkage of suspects to crimes. It is commonly collected from tools, weapons, clothing, and even human skin. However, collecting touch DNA presents challenges compared to other types of biological evidence due to various factors that can affect the amount of DNA collected [1]. These factors include the type of surface, collection and extraction methods employed [2], as well as the impact of time and environmental conditions [3]. In cases involving violent crimes like assault, sexual offenses, or homicide, touch DNA is frequently collected from the victim's clothes or their skin. However, there is a shortage of research exploring touch DNA collection methods specifically from human skin [4-5]. On the other hand, when collecting touch DNA from fabric, numerous variables come into play, including fabric type and area size, which can impact the collection process [6]. Additionally, the time interval between deposition and collection [7], as well as the method of collection [8], can also influence the success of touch DNA recovery from fabric.

The quality and quantity of touch DNA samples collected from the skin can be influenced by factors such as the applied pressure and duration of contact [9-10]. Various methods can be employed to collect touch DNA samples from the skin, with swabbing being the most effective and commonly used approach [10]. However, studies have indicated that flocked swabs are more effective than cotton swabs for collecting touch DNA from human skin [11], and the use of a wetting agent can enhance the efficiency of DNA recovery [12].

Direct PCR amplification is a valuable tool for improving DNA recovery [13]. It has been demonstrated that direct PCR amplification of touch DNA from human skin is a viable method for DNA analysis, generating DNA profiles of comparable quality to those obtained using conventional extraction methods [14]. However, the success of direct amplification of Touch DNA is contingent upon the quantity of cellular material present on the surface, which can be enhanced through the implementation of an efficient collection method [15]. Additionally, there are some observed effects associated with direct PCR, including increased stutter ratios, heterozygous allele imbalance, and split and shoulder peaks [16-18], which can pose challenges in interpreting data from mixtures of DNA profiles [19].

In manual strangulation, there is intense physical contact between the offender and the victim, thus the offender's epithelial cells are deposited on the victim's neck but the collection of touch DNA from the victim's skin can be complex because of the mixture of DNA present containing a small quantity of the offender's DNA compared to the victim's DNA. Validating different collection methods or techniques can improve touch DNA sampling [20-22], therefore, this study investigated three collection techniques involving cotton and nylon swabs to test their efficiency for the collection of touch DNA from the human neck.

Materials and methods

Experimental setup and deposition

Two male donors (perpetrators), previously identified as high and low shedders, were asked to wash their hands with antibacterial soap and refrain from any activity involving hand usage for 10 minutes. The neck skin of the receiver participants (male and female; victims) was disinfected using alcohol wipes (70% isopropyl alcohol), then cleaned with distilled water, and air-dried for 10 minutes. A donor was asked to hold the neck of the first receiver (male vs. female) as described in Figure 1. Previously, measurements were taken from three males and one female participating in the strangulation scenario of this experiment (Figure 2) for accurate sampling. After deposition, the neck was marked in three equal sections using a temporary marker pen for the recovery of touch DNA via three collection techniques. The DNA deposition was repeated for the other two participants (male vs. male). Furthermore, three recovery techniques were used to collect the randomly deposited DNA from the marked three sections to avoid using the same recovery technique for the same area. This was done to have a more effective sampling average for each technique used, as DNA amounts may shed differently from the hand of the donor during the physical contact in the strangulation process. Importantly, the deposition and collection processes were conducted at room temperature to avoid any environmental factors related to low or high temperature that can influence the skin such as sweating.

DNA recovery and extraction

A total of 48 samples were collected, with eight depositions made for each strangulation scenario (male vs. female and male vs. male) to obtain eight replicates for each collection technique.

After each deposition, touch DNA was collected immediately using a cotton swab (150C) (CS) (COPAN Diagnostics Inc.) and a nylon flocked swab (4N6 FLOQSwabs®) (NS) (COPAN Diagnostics Inc.), with three different collection techniques used as described in Figure 1. These techniques involved (a) moistening the swab with 100 µL of molecular grade water using a spray bottle for CS and moistening the swab with 30 µL of molecular grade water using a pipette for NS as recommended by the manufacturer, (b) using dry swabs, and (c) moistening the neck with 100 µL of molecular grade water using a spray bottle before collection with dry swabs.

Instead of using the pipette, a single swab technique was employed, with the cotton swab moistened using a spray bottle that was held approximately 25 cm from the swab (developed in the Dubai police forensic DNA lab) [16,19]. Each spray contained approximately 50 µL of solution, and the amount being sprayed was measured by spraying into an Eppendorf tube (1.5 mL) to confirm the amount.

The DNA was extracted immediately manually using a QIAamp® DNA Investigator Kit (Qiagen) following the manufacturer's protocol. Full swab heads were used for all DNA samples and a final extracted sample elution of 50 µL.

DNA quantification, amplification and analysis

Extracted samples were quantified using the Quantifiler® Trio DNA Quantification Kit and QuantStudio 5 Real-Time PCR (qPCR) and HID Real-Time PCR analysis software v1.3 (Thermo Fisher Scientific) according to the manufacturer's instructions. Next, DNA amplification was performed using the GlobalFiler™ PCR amplification Kit on a ABI GeneAmp® 9700 PCR System (Thermo Fisher Scientific) for 30 cycles following the manufacturer's protocol. Finally, amplified products were size-separated and detected on an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific) using 1 µl PCR product, 9.6 µl Hi-Di™ formamide, and 0.4 µl GeneScan™ 600 LIZ® Size Standard v2.0 (Thermo Fisher Scientific). The capillary electrophoresis products (STR data) were analysed using GeneMapper® ID-X Software Version 1.2, following the manufacturer's guidelines of the GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific).

Statistical analysis was performed with RStudio using factorial analysis of variance (ANOVA) and Microsoft Excel. In ANOVA, the p-value obtained from the F-distribution varied for each pair of degrees of freedom (df) values. The F value was calculated as the ratio of the variance of the variables' means (Mean Square Between) to the mean of the within variables' variances (Mean Squared Error). In this study, 'n' represents the total number of Touch DNA deposits or the number of samples collected.

The negative controls for the collection and extraction methods were DNA free when quantified and amplified. Control samples from the hands of the donors and the neck of the receivers were recovered after each sterilisation, which generated full single DNA profiles related to the participants without any sign of mixtures or contamination.

Results

By analysing the DNA quantities, a significant difference was observed between the three recovery techniques used to collect touch DNA with a cotton swab (CS) ($p < 0.05$) and a nylon swab (NS) ($p < 0.05$). For the CS, moistening the neck before collection (c) recovered more DNA than moistening the swab first (a) or using a dry swab (b) (mean a – 0.25, b – 0.37, c – 0.59 all in ng/µL; Figure 3). In contrast, for the NS, using a dry swab (b) recovered more DNA than moistening the swab first (a) or moistening the neck before collection (c) (mean a – 0.62, b – 1.02, c – 0.54 all in ng/µL; Figure 3), which is related to the neck skin being naturally moist and a nylon swab is more sensitive to moist surfaces than a cotton swab [20]. Nylon swabs (NS) recovered more DNA from the neck skin than cotton swabs (CS). This difference may be explained by the nature of the nylon fibers, which are harder compared to the soft cotton swab fibers [23]. When used on the skin, NS tends to be rougher, potentially enabling it to collect more cellular material from the victim's skin [16].

The touch DNA collected from the victim's skin usually contains a mixture of profiles comprising alleles from the victim's DNA and the perpetrator's DNA, so the amount of DNA does not necessarily lead to more alleles being recovered. All the collected samples from the victims in the strangulation scenario produced mixture profiles containing the DNA of the victims and the perpetrators. However, the number of alleles observed was not consistent ($p < 0.05$) among the collection techniques used. The number of alleles observed was much more consistent when neck skin was moistened before collection (c) for both swabs (CS alleles recovery a- 81%, b- 87% and c- 94% vs. NS alleles recovery a- 87%, b- 88% and c- 96%; Figure 4).

Discussion and Conclusion

Recovering trace DNA deposited on human skin poses greater challenges compared to collecting DNA from touched items due to the frequent occurrence of mixed DNA profiles. Consequently, collection methods play a crucial role in determining the quality of the collected DNA profile. Although cotton swabs are commonly used for trace DNA recovery, the amount of DNA retained by the swab can vary depending on the efficiency of the extraction method [1]. Employing an appropriate collection technique is therefore essential to enhance the quantity of DNA recovered from cotton swabs. Using a plastic spray bottle to moisten the swab is preferable to a pipette due to its ability to evenly distribute molecular-grade water without saturating the swab, reducing the risk of contamination [19]. However, it should be noted that the amount of water on the cotton swab may vary if the spray bottle is held at different distances from the swab prior to spraying [16]. Additionally, the quantity of solution sprayed by plastic bottles may differ, requiring careful consideration.

Furthermore, challenges can arise when recovering touch DNA from a victim's skin, including low DNA yield from the perpetrator and the risk of contamination [24]. These challenges highlight the importance of adhering to best practices in touch DNA analysis, which involve efficient recovery, proper collection, and meticulous handling of DNA samples, ensuring reliable and accurate results [25-26].

Several studies have explored the effectiveness of different collection methods for touch DNA recovery from human skin [4, 11-12]. While cotton and nylon swabs have demonstrated equal effectiveness in collecting touch DNA, SceneSafe Fast™ minitapes were found to be the least effective method [4]. However, the performance of swabs can be enhanced with the appropriate technique [19-20]. In the present study, three collection techniques were compared, revealing that moistening the neck with 100 µL of distilled water using a spray bottle before sample collection with a dry cotton or nylon swab significantly increased the allele recovery rate from the neck skin in the strangulation scenario. This finding underscores the importance of employing appropriate moistening techniques to optimise touch DNA recovery from human skin. It is important to consider that the recovery of touch DNA can be influenced by the age and condition of the skin, with older and drier skin yielding lower amounts of DNA [27]. Therefore, future studies should take these factors into account to advance our understanding of touch DNA recovery from human skin. Additionally, both manual and automated extraction methods have proven effective for touch DNA collected from human skin, yielding reliable and consistent DNA profiles, with magnetic bead extraction resulting in slightly higher DNA yields [28-29].

In conclusion, moistening the neck with 100 µL of distilled water using a spray bottle before sample collection with a dry cotton or nylon swab significantly increased the allele recovery rate from the neck skin in the strangulation scenario in this study. It is worth noting that the quantity of solution used can influence the amount of DNA collected; therefore, it is recommended to avoid exceeding 100-150 µL, which is commonly used to wet cotton swabs. This finding provides valuable guidance for optimising touch DNA collection in forensic investigations, particularly in cases involving strangulation.

Conflict of interest

None.

Acknowledgements

This study was approved by the General Department of Forensic Science and Criminology in Dubai Police and ethical approval was granted by the School of Forensic and Applied Sciences, and the University of Central Lancashire’s Research Ethics Committee (ref. no. STEMH 912).

Compliance with Ethical Standards

Funding: not applicable

Research involving Human Participants and/or Animals: not applicable

Informed consent: not applicable

Ethical Approval: This study was approved by the General Department of Forensic Science and Criminology in Dubai Police and ethical approval was granted by the School of Forensic and Applied Sciences, and the University of Central Lancashire's Research Ethics Committee (ref. no. STEMH 912).

Data Availability Statements: not applicable

References

1. Alketbi, S.K. (2018) The affecting factors of Touch DNA. Journal of Forensic Research, 9: 424.
2. Alketbi, S.K., Goodwin, W. (2019) The effect of surface type, collection, and extraction methods on Touch DNA. Forensic Science International. Genetics Supplement Series, 7(1): 704−706.
3. Alketbi, S.K., Goodwin, W. (2019) The effect of time and environmental conditions on Touch DNA. Forensic Science International. Genetics Supplement Series, 7(1): 701−703.
4. Kallupurackal, V. et al. (2021) Sampling touch DNA from human skin following skin-to-skin contact in mock assault scenarios—A comparison of nine collection methods. Journal of forensic sciences, 66(5), 1889–1900
5. de Bruin, K.G. et al. (2011) Comparison of stubbing and the double swab method for collecting offender epithelial material from a victim's skin. Forensic science international: genetics, 6(2), 219–223.
6. Alketbi, S.K., Goodwin, W. (2022) The impact of area size and fabric type on Touch DNA collected from fabric. Journal of Forensic Sciences & Criminal Investigation, 16(1): 555926.
7. Alketbi, S.K., Goodwin, W. (2022) The impact of deposition area and time on Touch DNA collected from fabric. Forensic Science International, Genetics Supplement Series, 8(1).
8. Alketbi, S.K. (2022) The impact of collection method on Touch DNA collected from fabric. Journal of Forensic Sciences & Criminal Investigation, 15(5): 555922.
9. Wallace, L., Merritt, R., & Carney, C. (2007). Collection and analysis of touch DNA deposited on skin. Journal of Forensic Sciences, 52(1), 1-7.
10. Oldroyd, N. J., Watson, S. K., & Miller, K. (2017). Collection and analysis of touch DNA: A practical guide. Forensic Science International: Genetics Supplement Series, 6, e595-e596.
11. Jang, C. G., Park, J. H., & Lee, H. C. (2015). Comparison of two swabbing methods for the recovery of touch DNA from human skin. Forensic Science International: Genetics, 14, 247-252.
12. Hogan, C., Oldroyd, N., & Miller, K. (2019). Comparison of two methods for the collection of touch DNA from human skin. Forensic Science International: Genetics Supplement Series, 7(1), 49-50.
13. Alketbi, S.K. (2022) An innovative solution to collect Touch DNA for direct amplification. Journal of Forensic Sciences & Criminal Investigation, 16(1): 555928.
14. Chong, E. K., & Chan, K. L. (2017). Direct PCR amplification of touch DNA from human skin. Forensic Science International: Genetics Supplement Series, 6, e533-e535.
15. Alketbi, S.K., Goodwin, W. (2023) Collection Methods for Touch DNA Direct Amplification. Journal of Forensic, Legal & Investigative Sciences, 9: 072.
16. Alketbi S.K. (2023) Analysis of Touch DNA. Doctoral thesis, University of Central Lancashire.

17. Gouveia, N., Brito, P., Serra, A., Balsa, F., Andrade, L., Bento, M.S., Cunha, P., Bogas, V., Lopes, V., Porto, M..J. (2015) Direct amplification of reference samples with Globalfiler® PCR Amplification Kit. Forensic Science International: Genetics Supplement Series, 5, pp. e135–e137.

18. Ambers, A., Wiley, R., Novroski, N. and Budowle, B., (2018). Direct PCR amplification of DNA from human bloodstains, saliva, and touch samples collected with microFLOQ® swabs. Forensic Science International: Genetics, 32, pp. 80-87.

19. De Jong, W. K., & Kleibeuker, K. H. (2019). The forensic potential of touch DNA on human skin. Forensic Science International: Genetics Supplement Series, 7(1), 31-33.

20. Alketbi S.K., Goodwin. W. (2019) Validating Touch DNA collection techniques using cotton swabs. Journal of Forensic Research, 10: 445.

21. Alketbi, S.K., Goodwin, W. (2021) Touch DNA collection techniques for non-porous surfaces using cotton and nylon swabs. Biomedical Journal of Scientific & Technical Research, 36(3): 28608−28612.

22. Alketbi, S.K., Goodwin, W. (2019) The effect of sandy surfaces on Touch DNA. Journal of Forensic, Legal & Investigative Sciences, 5: 034.

23. Squassina A, Piovanelli E, Castriciano S. (2014) Evaluation of DNA recovery from Copan Genetics FLOQSwabs™ for human identification; 25th International Symposium on Human Identification Poster Abstracts. Available at: https://www.promega.com/-/media/files/resources/conference-proceedings/ishi-25/poster-abstracts/18-alice-squassina.pdf (Accessed: 11 June 2022).

24. Ruff, P., & Holtkötter, H. (2017). Forensic investigation of trace DNA from human skin. Forensic Science International: Genetics Supplement Series, 6, e415-e417.

25. Alketbi, S. K. (2023) Maintaining the chain of custody: Anti-contamination measures for trace DNA evidence. International Journal of Science and Research Archive, 08 (02). pp. 457-461.

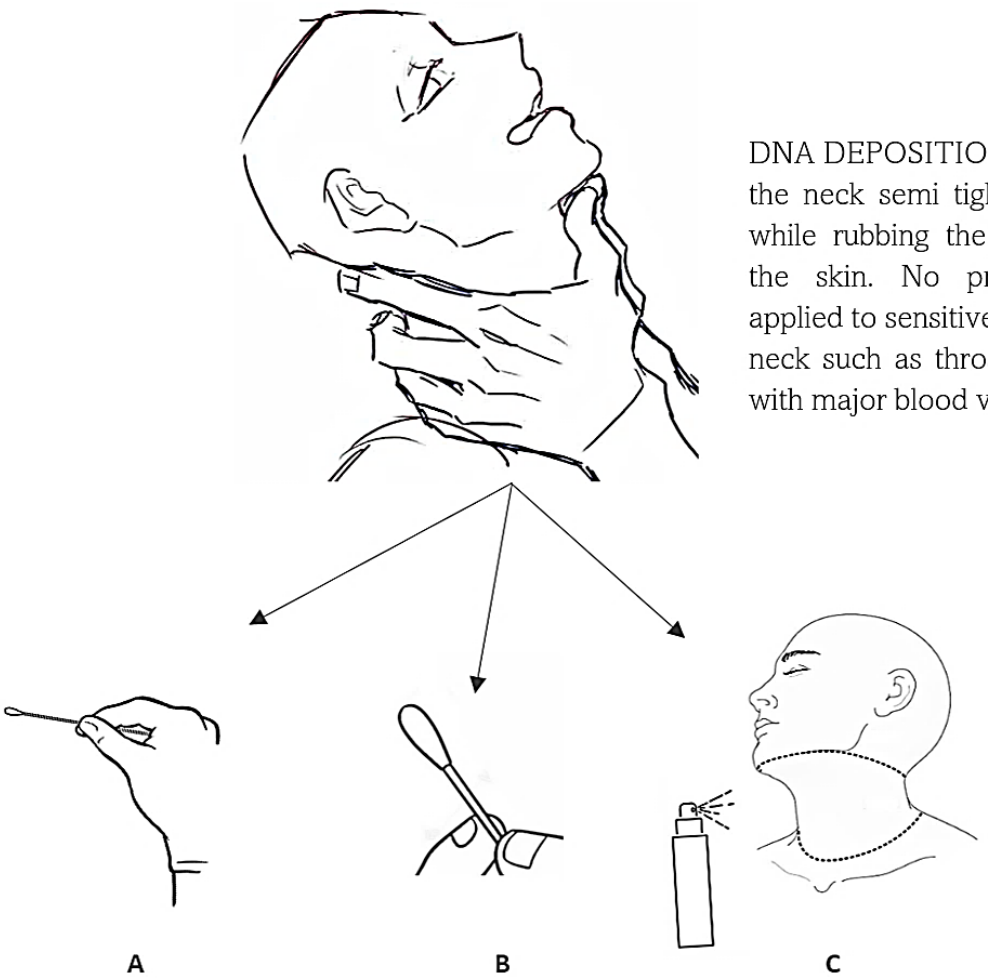
26. Sijen, T. (2015). Touch DNA evidence: From crime scene to court. Human Genetics, 134(3), 259-271.

27. Van den Berge, M., Bhoelai, B., Harteveld, J., & Matai, A. (2019). Touch DNA recovery from human skin. Forensic Science International: Genetics Supplement Series, 7(1), 52-53.

28. Bleka, Ø., Haugum, M., & Gill, P. (2014). A comparison of DNA profiles produced from automated and manual extraction from human skin and contact DNA samples. Forensic Science International: Genetics, 9, 29-34.

29. De Rijke, B., & Westen, A. V. (2021). Touch DNA analysis on human skin: Method comparison for swabbing and extraction. Forensic Science International: Genetics, 53, 102497.

DNA COLLECTION via three techniques (A) moistening the swab with 100 µl of distilled water using a spray bottle for CS; moistening the swab with 30 µl of distilled water using pipette for NS (B) dry swab and (C) moistening the neck with 100 µl of distilled water using spray bottle before collection with dry swabs.



DNA DEPOSITION by holding the neck semi tight for 2 min while rubbing the hands over the skin. No pressure was applied to sensitive areas of the neck such as throat and areas with major blood vessels

Figure 1 – DNA deposition to mimic a strangulation scenario and collection of Touch DNA from neck skin using three collection techniques (A, B and C) with a cotton swab (CS) and a nylon swab (NS).

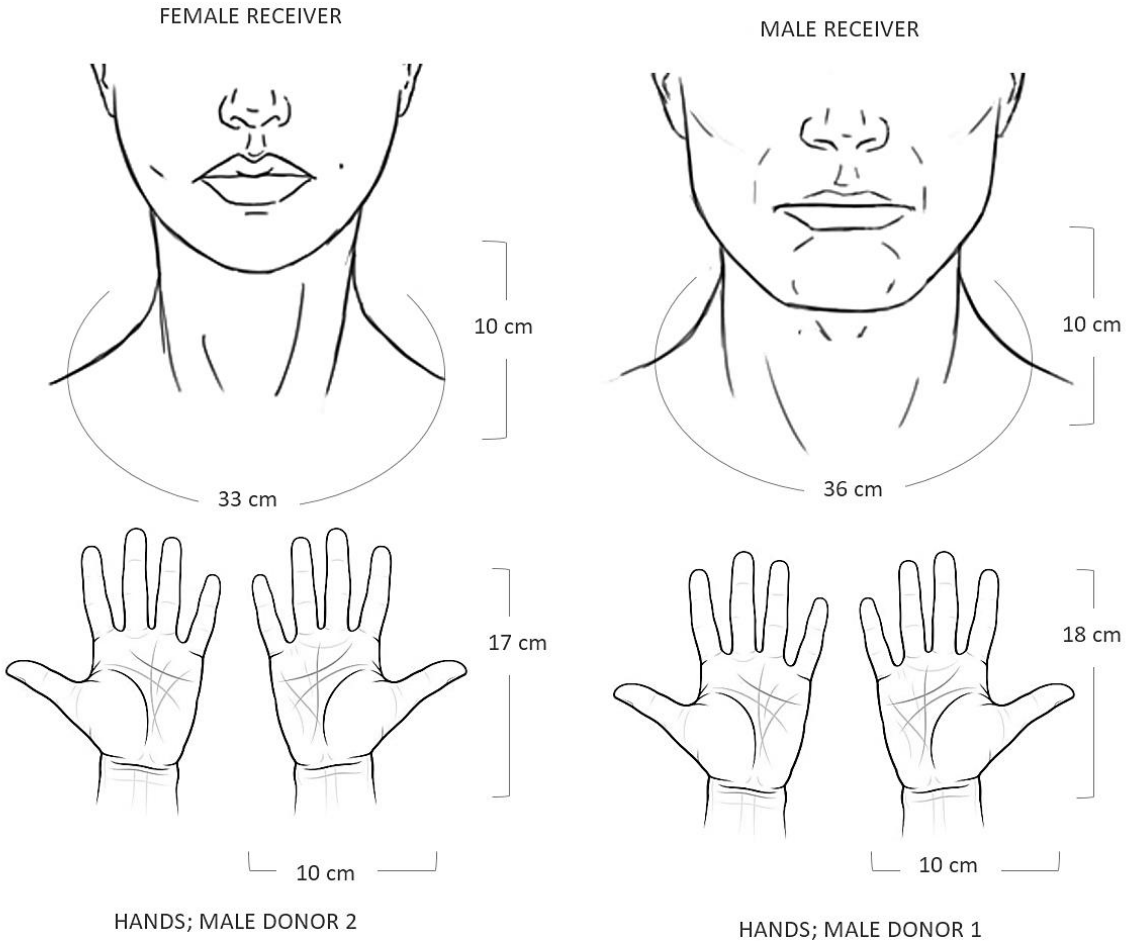


Figure 2 – Measurements of the hands and necks were taken from the participants involved in the strangulation scenario, male donor vs. female receiver and male donor vs. male receiver. The hand measurements of the donors were very similar, whereas the neck measurement of the male receiver was 3 cm larger in circumference than that of the female receiver. To ensure accurate sampling from the neck, it was marked into three equal sections, which were used for collection with three different techniques after each DNA deposition. For the male vs. female scenario, the section size was 11 cm x 10 cm, and for the male vs. male scenario, it was 12 cm x 10 cm."

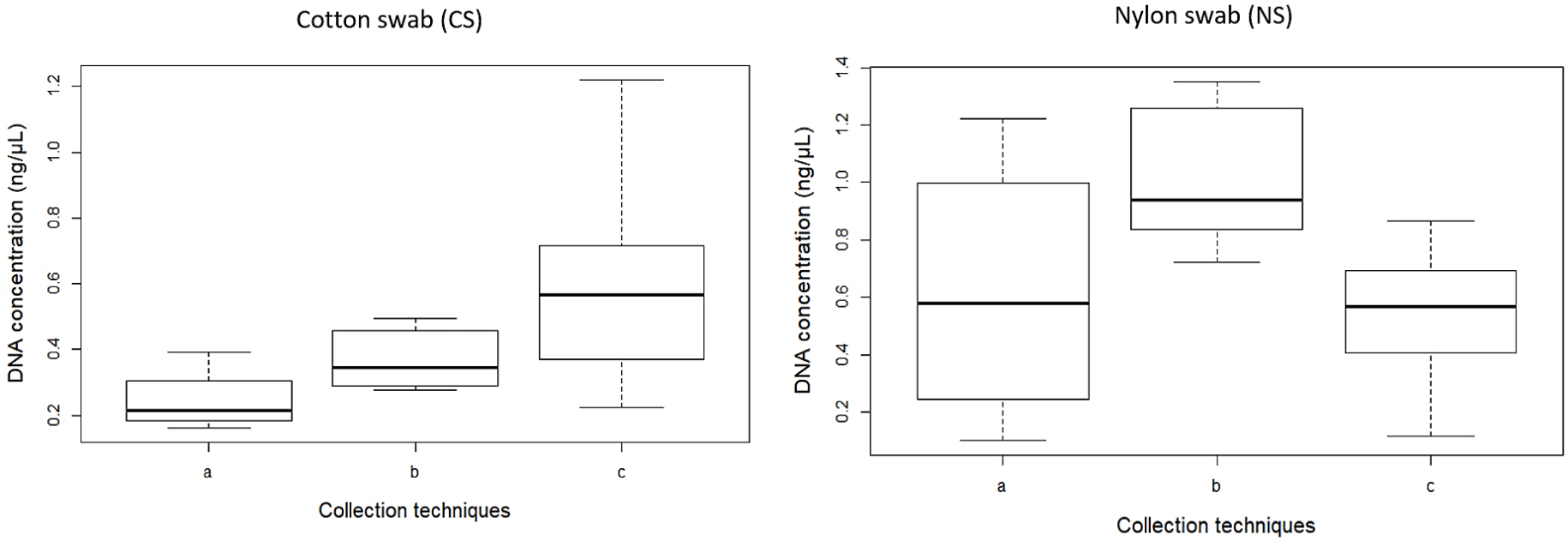


Figure 3 - The mean DNA recovered (n= 48) from neck skin by the three techniques using a cotton swab (CS) and nylon swab (NS): (a) moistening the swab with 100 µl of distilled water using a spray bottle for CS and moistening the swab with 30 µl of distilled water using pipette for NS, (b) dry swab and (c) moistening the neck with 100 µl of distilled water using spray bottle before collection using dry swabs. There was a significant difference between the three recovery techniques used to collect touch DNA with a cotton swab (CS) ($p < 0.05$) and a nylon swab (NS) ($p < 0.05$). For the CS, the mean values were as follows: a – 0.25 ng/µL, b – 0.37 ng/µL, and c – 0.59 ng/µL. As for the NS, the mean values were a – 0.62 ng/µL, b – 1.02 ng/µL, and c – 0.54 ng/µL.

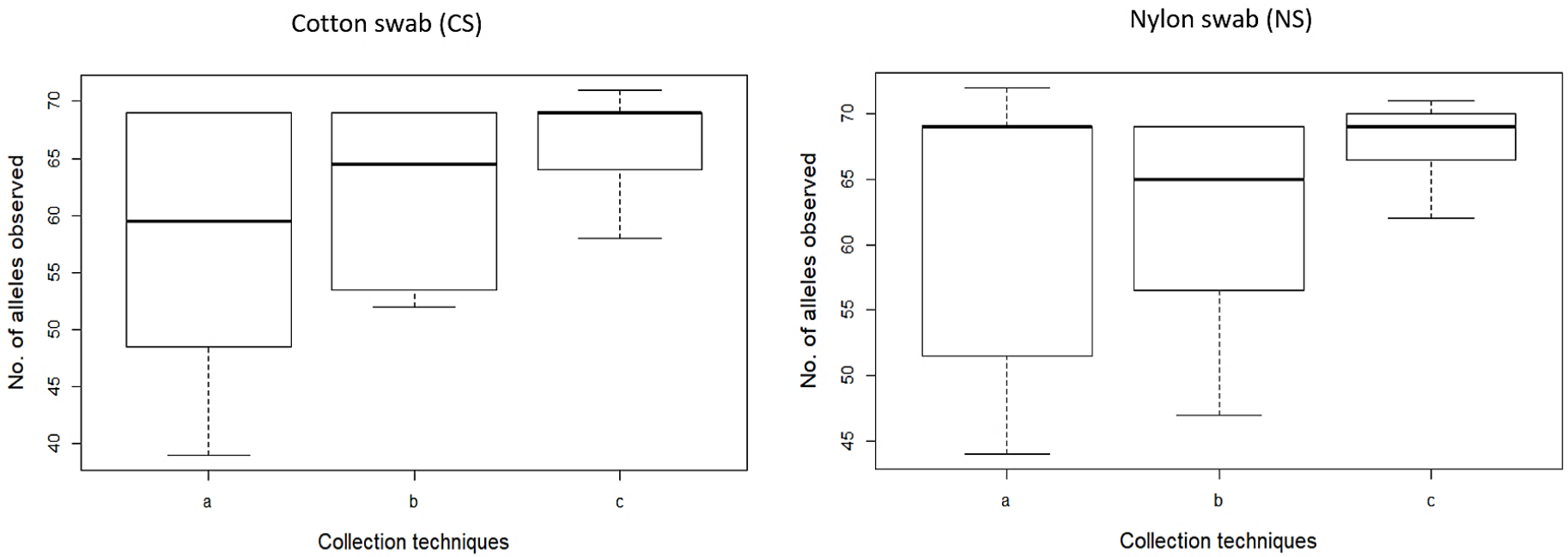


Figure 4 - Number of alleles observed (n= 48) for each technique using a cotton swab (CS) and nylon swab (NS): (a) moistening the swab with 100 µl of distilled water using a spray bottle for CS and moistening the swab with 30 µl of distilled water using pipette for NS, (b) dry swab and (c) moistening the neck with 100 µl of distilled water using spray bottle before collection using dry swabs. From the participants involved in the strangulation scenario, the total number of alleles observed in full mixed DNA profiles were 72 alleles for male donor vs. female receiver and 69 alleles for male donor vs. male receiver. All the collected samples produced mixture profiles, but the number of alleles observed was not consistent ($p < 0.05$) among the collection techniques used. For CS alleles recovery, the percentages were as follows: a - 81%, b - 87%, and c - 94%. On the other hand, for NS alleles recovery, the percentages were a - 87%, b - 88%, and c - 96%.