Legal Medicine 81 (2026) 102803

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Preliminary study of mixed traces on cigarette butts and non-self DNA transfer, persistence, prevalence and recovery in different forensic scenarios☆

Denise Gianfreda[a,*], Beatrice Corradini[a], Gianmarco Ferri[a], Francesca Ferrari[b], Ilaria Borciani[a], Rossana Cecchi[a], Anna Laura Santunione[a]

[a] *Institute of Legal Medicine, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Via del Pozzo - 71, 41125 Modena, Italy*

[b] *Institute of Legal Medicine, University Hospital of Modena, Via del Pozzo - 71, 41125 Modena, Italy*

A R T I C L E I N F O

Keywords:
Saliva traces
Activity level proposition
DNA-TPPR
Cigarette butt evidence
DNA mixture
Kissing
Smoking

A B S T R A C T

Recent studies focus on the different ways in which DNA can be transferred from and individual to another or to a surface.

This work investigates how mixed DNA profiles can be generated on items commonly collected at the crime scene, specifically on cigarette butts. The aim is to determine how biological material exchanged during intimate contact (kissing or sharing a cigarette) can be secondarily transferred onto cigarettes, and how long non-self DNA persists and remains recoverable under different conditions and time intervals.

The preliminary experimental study involved one investigative couple: each volunteer smoked a cigarette at fixed intervals after kissing (from 0 to 120 min) and both partners smoked a shared cigarette.

DNA was extracted from cigarette butts immediately and after 30 days using the QIAamp DNA Investigator Kit on the QIAcube Connect. Quantification was performed with PowerQuant® System Kit and genetic profiles were obtained using PowerPlex® Fusion and Y23 Kits.

Results showed non-self DNA alleles up to 2 h after kissing, even in non-immediate extractions, partial Y-STRs profiles in female butts after 1 h: individual variability and degradation made interpretations harder. Based on preliminary findings, the study will be expanded including more partner pairs, more smoking devices and longer processing timeframes.

Our findings suggest the possibility of reconstructing transfer scenarios leading to mixed DNA traces on cigarette butts and of assessing a time window within which non-self DNA can be secondarily transferred to the cigarette, highlighting the impact of processing times and smoking device on DNA results.

1. Introduction

Evaluation of DNA traces given activity level propositions is of growing interest in court. The increase in the sensitivity of forensic genetics analytical techniques, instruments and methods allow the recovery of complete DNA profiles from just a dozen of intact cells. Advantages of this lie in the possibility of obtaining many more DNA results from even very spotty and deteriorated traces, however these require a much higher and advanced level of interpretation.

These improvements certainly benefit investigations, allowing scientific leads to be provided in the most complex cases, however, communicating the results to prosecutors and jury is complicated, leading to miscarriage of justice if DNA results are not proper handled by forensic experts. It is increasingly important for scientists, lawyers, prosecutors and judges to recognize the limitations of DNA profiling evidence [1].

Recently studies have examined the different ways in which DNA can be transferred from one individual to another, or from an individual to surfaces and objects, bi-directionally as well [2–4]. DNA is transferred by hand contact or through body fluids, like by saliva or semen

---

☆ This article is part of a special issue entitled: ‘Proceedings Congress Forensic Science’ published in Legal Medicine.

* Corresponding author.

E-mail address: denise.gianfreda@unimore.it (D. Gianfreda).

https://doi.org/10.1016/j.legalmed.2026.102803
Received 6 August 2025; Received in revised form 9 January 2026; Accepted 1 February 2026

exchange. When DNA is deposited through direct contact, this is referred to as primary transfer, when it is deposited through subsequent indirect contacts, this is referred to as secondary transfer [3].

Interpreting a genetic profile is no longer just about answering the question “Who is the donor of the trace?” from an evaluative point of view, but also about answering questions as “By which biological fluid has been the DNA transferred? How? By what activities? Are these related to the crime?”, in an investigative approach [5,6]. There are many situations in which doing so is essential, and most of this concern the interpretation of mixed traces. Evaluation of biological findings given activity level propositions (i.e. compare probabilities of DNA results given at least two mutually exclusive propositions regarding disputed activities that might have resulted in the evidence) is therefore crucial [7] and, as emphasized in the International Society for Forensic Genetics (ISFG) commission recommendations, it represents the most reliable investigative operational approach, both intellectually and scientifically [5,6].

Understanding factors such as time and material/surface composition that affect DNA transfer, persistence, prevalence and recovery (DNA-TPPR [3]) is of importance for evaluating how the DNA of a person is present in forensic evidence. Experimental studies are essential to support accurate interpretation in real case scenarios.

The study was inspired by a prosecutor's inquiry into whether any literature data was available on the persistence and recovery of mixed saliva traces on cigarette butts. There is a notable lack of scientific literature concerning mixed DNA traces on this type of evidence and consequently data on the interpretation of these findings considering activity level evaluations are also scarce. Scenarios involving intimate contacts between partners are frequent in cases of homicide, sexual assault or gender-based violence of subjects with a sexual or romantic intercourse.

Cigarette butts are widely collected during crime scene inspections as evidence and hence subjected to DNA profiling [8,9,41]. Statistical analyses, considering activities resulted in mixed saliva traces on this item can be extremely complex. Saliva is frequently spotted in forensic contexts and can be vehicle for DNA transfer between people and objects, researches have examined how it behaves on different conditions and surfaces [10–16].

Cigarette may have been smoked by two individuals alternating or smoked immediately or after a certain time interval after kissing. Other factors may complicate DNA profiling analysis and interpretation of this type of evidence, leading to a progressive loss and deterioration of DNA; for instances the possibility of discovering in outdoor or indoor environments (adverse conservation modes and timing) and prolonged time intervals between sampling and processing.

In the case mentioned, a suspect was identified as victim offender following multiple investigative leads, including a cigarette butt found at the crime scene. A mixed DNA trace matching victim’s and suspect’s DNA was detected on a cigarette butt retrieved at the crime scene, where the woman's body was discovered. According to the suspect’s account, he was no longer with the victim at the time of the crime, suggesting that the victim’s DNA may have remained in his mouth and been transferred to the evidence when he smoked the cigarette.

The study of Banaschak et al. [17] is the first literature contribution that investigated the possibility of a DNA mixture of saliva samples after intensive kissing. Subsequently, Kamodyovà et al. [18] have extended examinations aimed at analysing prevalence and persistence of male DNA in female saliva samples evaluating the same post-kiss intervals. Both studies [17,18] employed techniques whose sensitivity and specificity are no longer representative of current forensic standards and technologies, resulting in limitations that make their applicability as a reference more challenging for recent cases evaluations at activity level. Some of these drawbacks have been addressed by the recent study of Pesaresi et al. [19]. The authors using current forensic technologies analysed various areas of the oral cavity (perilabial, labial, teeth, and tongue regions) to assess the persistence of exchanged DNA up to 120 min after kissing, highlighting the forensic relevance of specific sampling sites.

Although therefore study have investigated DNA-TPPR phenomena of saliva mixed traces after partners kiss, none of these, to the best of authors knowledge, assessed how exogenous DNA existing in mouths can be secondarily transferred onto another surface, as that of a cigarette filter.

The aim of the research is therefore to deepen the understanding of the activities leading to mixed genetic profiles on cigarette butts and investigate legal implications of such findings. For this purpose, pairs of volunteers will be recruited to simulate two scenarios, easily verifiable in forensics routine, involving DNA exchange between individuals themselves and different cigarette devices such industrial and filtered electronic cigarettes. Qualitative and quantitative analysis will be carried out, autosomal and Y-chromosome markers will be typed to assess partners proportion and statistical value of their contributions to the mixed the trace.

2. Materials and methods

The study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All procedures were performed in compliance with current legislation, all personal data collected will be processed in respect of privacy. Genetic data and biological samples are anonymized using alphanumeric codes, in accordance with ethical and legal regulations on the handling of biological samples and personal data. The project was approved by Research Ethics Committee of the University of Modena and Reggio Emilia (CEAR) registered 2025-UNMRCLE-0073656 on 27/02/2025, all volunteers given informed consent for participation and to provide reference and experimental samples.

Pilot study presented involves one investigative couples of smoker partners who were asked to simulate the two experimental scenarios and collect reference buccal swabs. Volunteers were hand-rolled self-prepared cigarettes smokers. For the first scenario (Scenario 1) a couple was asked to simulate an experiment in which each partner smoked a cigarette 0, 5, 15, 30, 60, 90 and 120 min after a French kiss and for the second scenario (Scenario 2) another couple was asked to half-smoking a shared cigarette by passing it in turn. Finally collect reference samples through buccal swabs.

The pilot study involves 30 cigarette butt samples.

Cigarette butt samples were processed immediately and after one month, to estimate if processing time could impact DNA results.

Reference DNA collected with 4N6FLOQSwabs® Genetics (Copan) was extracted from buccal cells using QIAamp DNA Mini QIAcube Kit (Qiagen) with ‘blood and body fluids’ protocol diluting the swab in 1 ml and resuspending the cell pellet in 200 μl of sterile water.

DNA from cigarette butt samples was extracted on QIAcube Connect (Qiagen) with the dedicated lysis and purification ‘forensic casework samples’ protocols of the QIAamp DNA Investigator Kit (Qiagen) to assess applicability of automation in terms of DNA quantity and quality. The filter pater was sectioned and placed directly on the instruments; DNA was eluted in 40 μl of ATE buffer. Quantity, degradation, inhibition and male–female ratio evaluations were carried out through Real-Time quantitative PCR (qPCR) with PowerQuant® System Kit (Promega) on 7500 Real-Time PCR System (Applied Biosystems), data analyzed with PowerQuant® Analysis Tool v1 Microsoft Excel macro file (Promega).

A range of 0.5–1 ng/μl of DNA was amplified (if quantification did not reach this range, the maximum volume available was used as PCR template) by adding 0.2 μl (20 mg/mL stock solution) of Bovine serum albumin (BSA), DNA profiling was performed with PowerPlex® Fusion and Y23 Kits (Promega) on SeqStudio Genetic Analyzer for HID (Applied Biosystems), electropherograms analysed with GeneMapper ID-X v 1.6 (Applied Biosystems) by using 50/150 RFU analytical/stochastic threshold. A profile with more than 11 unambiguous loci was considered suitable for comparisons [20].

Statistical analyses were performed with Microsoft Excel and RStudio. The Shapiro-Wilk test was used to test data for normal distribution requirement, hence either a non-parametric test or the *t*-test was applied.

DNA profiles comparisons and discriminative assessment analysis were carried out with LRmixStudio software [21]: shared alleles between partners and alleles in stutter position were excluded from the non-self-allele account used for transfer, persistence, prevalence and recovery analysis. The DNA of one of the partners, who had only kissed the person who smoked the cigarette, was considered a non-self when it was found on the cigarette butt.

Mixture proportion evaluations were performed with DNAstatistX software with default settings, LR values above 1000 threshold may be considered sub-source level informative [22,23].

3. Results

3.1. DNA extraction and quantification

Automatic DNA extraction from cigarette butt samples yields positive DNA amount. Details of PowerQuant® System Kit (Promega) results referring to the autosomal and Y-chromosome DNA, male–female proportion, degradation and inhibition indices are detailed in Supplementary Table 1.

Quantitative data show that the [Auto]/[Y] ratio is significantly higher than 1 in cigarettes smoked by female, while it remains around 1 in those smoked by male. Conversely, the ratio tends to stay close to 1 in the male cigarettes.

The internal PCR control (IPC) shift values were in average below the manufacturer's recommended threshold, indicating no significant inhibition caused by the cigarette composition materials.

Trends in autosomal DNA concentration and degradation index over the two processing times (immediate and + 30 delayed) obtained from the 30 samples analysed are summarized in Fig. 1 and Fig. 2 respectively. Quantification data suggest a downward trend in DNA extracted with a statistically significant decrease in DNA quantity between the initial extraction and the delayed one (Wilcoxon signed-rank test, p = 00037), as well as a statistically significant increase in degradation index (Paired one-tail *t*-test, p = 0.00209). indicating a likely loss and DNA degradation over time.

3.2. DNA profiling

Complete DNA profiles were obtained for all cigarette samples processed immediately, at least all alleles referable to the smoker's profile were detected. Partial profiles were detected in samples processed after 30 days, allelic drop-out also affected alleles of the direct smoker.

3.2.1. Scenario 1

Reference profile comparisons allow for the identification of autosomal STRs unique alleles, i.e. alleles not shared between male and female partners [19,24–30].

Among the total of 27 alleles for the female and 21 for the male (homozygous alleles counted as one), after excluding alleles in stutter positions, 9 alleles can be considered discriminative for the female and 10 for the male and used for non-self allele detection in mixed profile analyses.

The contribution of each donor recovered in the mixed trace appears to reflect the type of transfer occurred: the major contributor is that of the person who directly smokes the cigarette (i.e., primary transfer), while the minor contributor is that of the partner whose DNA was transferred via the prior kiss (i.e., secondary transfer).

The non-self contributions detected slightly differed between cigarette samples smoked by male and female as can be seen in the differences between the discriminative alleles recovered from male and female cigarette samples.

Fig. 3 provides a detailed view of the number of autosomal discriminative non-self alleles recovered at various time points after the kiss, for both male and female cigarettes, and for both the processing times. It shows a slight downward trend: as the time between the kiss and cigarette smoking increases, the number of detectable non-self alleles decreases. DNA from the kissing partner is still detectable 2 h after the kiss, however, allegedly the minority component gradually decreases to disappears after two hours.

Supplementary Table 1 presents the number of unique non-self alleles, including stutter, and the percentage of the partner's profile recovered in mixed DNA traces. These data further support the persistence of secondary transferred DNA up to two hours after the kiss.

Additionally, the results indicate that the processing time influenced non-self DNA recovery, with delayed processing generally yielding lower amplification of the non-smoking partner's DNA component.

Y-STR typing of female cigarette butt samples immediately processed yielded complete profiles suitable for comparative analysis for smoking time intervals ranging from immediately after the kiss up to one hour later. In the last time interval (2 h), a marked decrease in the number of detected Y alleles was observed; however, the male component remained detectable. Fig. 4 shows the number of Y alleles recovered in

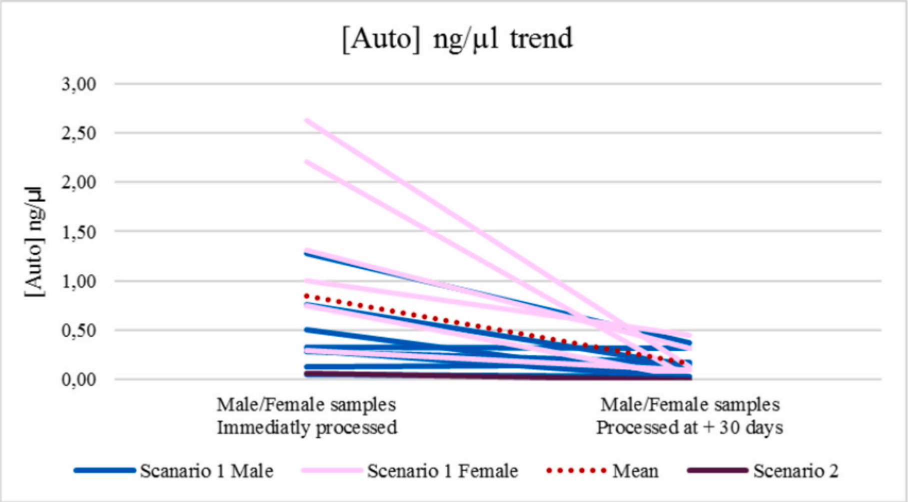

Fig. 1. Autosomal DNA quantified in the first immediate extraction and in the second delayed of 30 days.

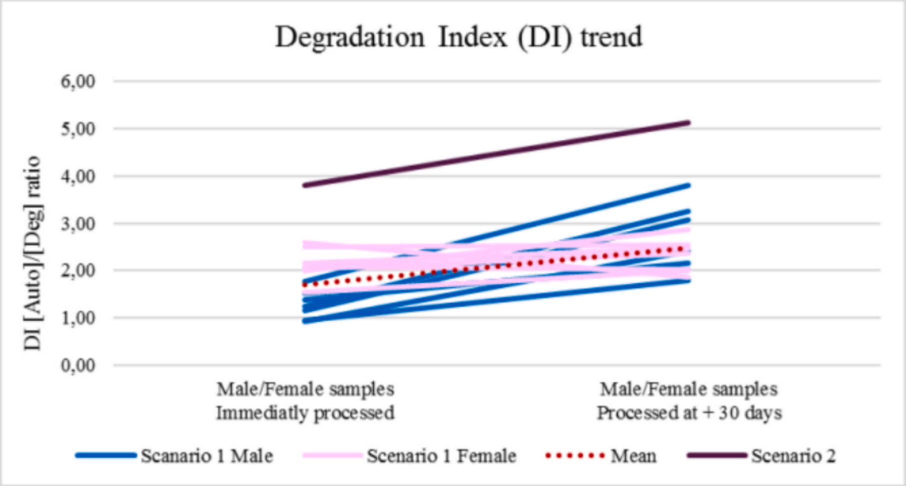

Fig. 2. Degradation Index measured in the first immediate extraction and the second delayed of 30 days.

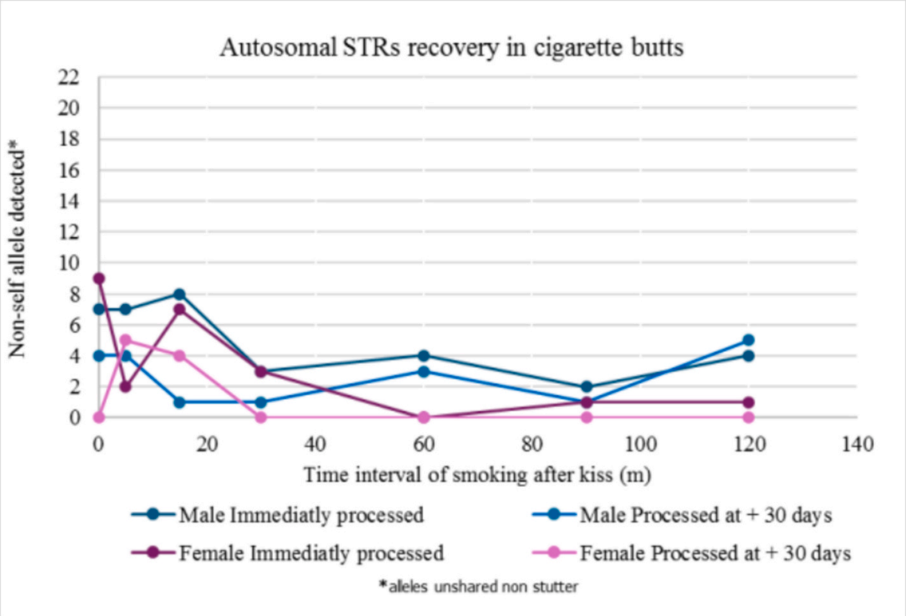

Fig. 3. Autosomal STRs discriminative alleles detected in male and female cigarette butt smoked at set time point after kissing.

each sample both in immediate and delayed processing. In samples extracted 30 days later, the male contribution was lower, but alleles attributable to the male partner were still detectable up to the two-hour post-kiss smoking interval.

3.2.2. *Scenario 2*

DNA profile obtained from the shared cigarette half-smoked by each partner was a quite well-balanced mixed profile, reflecting the assumption that both partners transfer their DNA through saliva exchange on direct primary transfer. Allele recovery was of 100% for the male donor and 93% for the female in sample immediately processed, the percentage refers to ratio between the total alleles of the donor profile and the corresponding peak detected in the mixture.

DNAStatistX results in a 76%-24% mixture proportion in which male DNA is the major contributor.

In sample analysed with 30 days of delay, allelic drop-out for both contributors were observed. The partial trace did not display a sufficient number of markers for statistical comparison; hence the mixture proportion evaluation was not carried out.

4. Discussion

Evaluate DNA results given activity level propositions is an important recommendation from ISFG [5,6] for forensic geneticists necessary to ensure impartiality. Nowadays is widely recognised the importance of set up standardized experimental study under conditions relevant to criminal cases aimed at build, refine knowledge and sharing data about DNA transfer, persistence, prevalence and recovery events [23,31–33]. Research work are crucial to understand the many variables that can impact DNA evidence and support probability assignments. Many elements can affect the results: direct or indirect transfers, physical and chemical differences of contacting surfaces, deposits and processing time periods, multiple contributors’ proportion and subjective variability in DNA release [31,32,34–36].

This work presents preliminary results from an experimental study which aimed to address a gap in the literature on mixed DNA traces and potential DNA-TPPR phenomena on cigarette butts.

The automatic extraction procedure yields good amount of DNA from a single slice of cigarette butt filter paper. These techniques prove

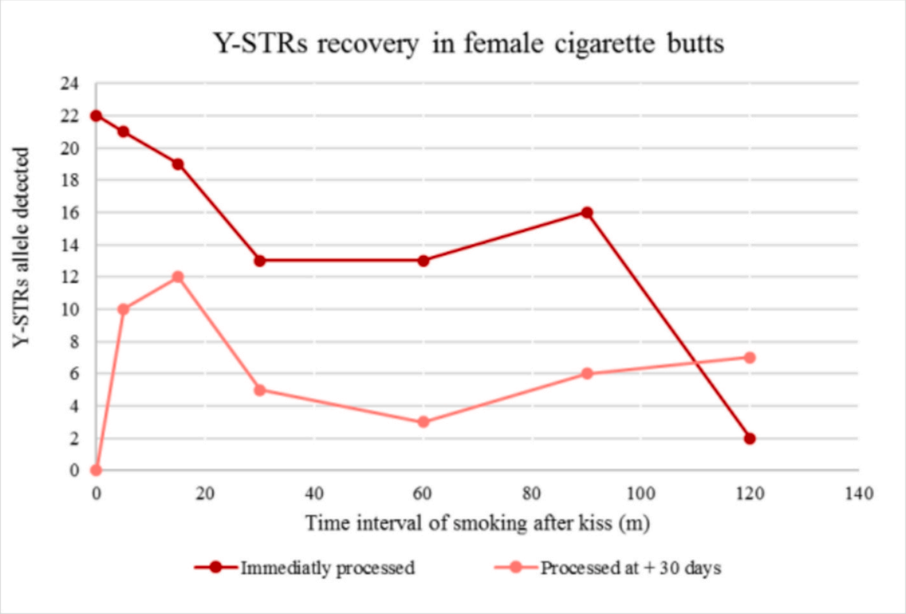

Fig. 4. Y-chromosome STRs, referable to male partner, detected in female cigarette butt smoked at set time point after kissing.

to be a good choice in terms of standardisation, essential for the scope of the study, limitation of manual handling (decreasing the risk of contamination) and optimisation of time and work sessions, important feature in the forensic field, especially for scenarios where a high number of samples must be processed in a short period of time.

A sufficient amount of DNA to perform subsequent amplification analyses was obtained from all samples immediately processed. The different quantity extracted from each cigarette sample smoked by the same individual may be due to several accidental factors: fluctuating saliva content during the day [14], different salivary release, different pressure of the cigarette between the lips, different number of times the cigarette was brought to the mouth to inhale.

No significant differences were noted between the DNA extracted from butts of cigarettes smoked by female and male in terms of quantity. Although an inter-individual variation is certainly present, the preliminary results are insufficient to note differences in salivary production and transfers between the two sexes, as highlighted in previous works [19,37]. Pesaresi et al. [19] observed a pronounced allelic disparity between male and female perilabial samples, with male DNA persisting on females more consistently than the reverse. This discrepancy should also be reflected in the secondary transfer of non-self DNA from the perilabial female area to the cigarette filter area; however, such defined pattern was not observed in our pilot study. Fig. 3 would suggest almost the opposite.

Nevertheless, it should be emphasized that these considerations can only be taken into account for samples extracted immediately, as this analysis procedure can be considered the most reliable, and which must be confirmed by a larger number of couples analyzed.

From a technical point of view, cigarette samples are known to contain inhibitors for PCR amplification reactions [8,38], despite this, the IPCswift values resulting from the quantitative analysis were on average below the threshold. All amplifications were positive and in agreement with the amount of DNA extracted and its degree of degradation. However, to enhance PCR performance, BSA was added, which is known to be a favorable factor for the reaction [39].

Comparison between reference profiles and mixed traces indicates that as the time between the kiss and cigarette smoking increases, the number of detectable non-self alleles decreases, while retaining a persistence detectable 2 h after the kiss, both in male and female cigarette. In most samples, the minor alleles were enough to statistically support the non-self donor contribution through likelihood ratios calculations, showing the usefulness of these results in potential forensic contexts (see Supplementary Table 1 material).

Interpretation of these mixed DNA profiles, particularly those involving a low-level minor contributor, was carried out using a continuous probabilistic genotyping approach, hence considering peak height information and associated stochastic effects, this framework allows the strength of the evidence to be evaluated while explicitly accounting for uncertainty, thus avoiding overinterpretation of partial profiles.

The quantification data relating to immediate and 30-day postponed processing suggests a loss and degradation of DNA over time. There is a decrease in the amount of DNA recovered in 30-day postponed processing, accompanied by an increase in the degradation index. These issues impacted most noticeably on non-self DNA, being the minority component initially in smaller and sub-optimal quantities, leading to a decrease in detectable alleles with a consequent loss of information on non-self DNA persistence.

Studies indicates that when saliva mediates DNA transfers, the primary depositor generally dominates DNA mixture showing the primary donor as the major contributor [10,15], this was also observed in the Scenario 1 samples of the presented study (Supplementary Table 1). In samples of the Scenario 2, where both partners serve as primary source smoking the half-shared cigarette, the situation arises was also observed, without, however, leading to a perfectly balanced mixture (76% male – 24% female). The explanation for the imbalance could lie in the type of device used for the smoke experiments. Participants were hand-made tobacco cigarette smokers, this type of device requires the tobacco, together with the filter, to be rolled into a paper wrapper which will be closed by licking an end containing glue. In the case, the male counterpart prepared the cigarette, therefore, allegedly this could be the reason for the higher male contribution in the mixture of second scenario samples. In the cigarettes in the first scenario, same type of device, this could probably have been masked by the fact that the exogenous contribution came from an indirect transfer and was therefore already smaller in quantity.

The ratio between the amount of autosomal DNA, which includes both male and female contributions, and that of Y-chromosome DNA reveals a marked difference between female and male cigarette samples. Moreover, in female samples, as the kissing-smoking time interval increases, the ratio tends to rise proportionally, presumably as a consequence of decreased Y-chromosome detection. Therefore, the values of the ratios may be indicative of the smoker, at least of the partner who contributed most to the mixture.

Amplification of the specific Y-STRs markers enables to deeply evaluate the contribution and persistence over time of the male counterpart in female cigarette samples, yielding reliable profiles one hour after kissing, with some alleles still detectable up to two hours later. Data are in accordance with the findings of colleagues Pesaresi et al. [19] and Kamodyovà et al. [18]: due to the technologies sensitivity, the detectable persistence times are such, even considering the indirect transfers occurring in the presented work.

The study provided important insights into phenomena associated with mixed DNA traces on cigarette butts, although limited to a single couple, and laid the groundwork for expanding the sample size and refining the study design including a longer kissing-smoking time interval, more devices (traditional and e-cigarette) and two different (closer and stretched) processing timings.

This works highlights the importance of considering evidence material composition and degradation, when dealing with biological findings evaluations given activity level propositions.

Finally, the further study will follow minimum requirements for publishing data on DNA-TPPR given activities and checklist provided recently by Gill et al. [23,40] to assure standardization of the laboratory procedure, consistency in reporting results and facilitate application in real legal proceedings.

5. Conclusion

Interpreting mixed traces resulting from indirect contact can be a complex task, especially when the activities leading to their deposition are disputed and the contribution of non-self DNA is in question.

Our findings suggest the possibility of reconstructing transfer scenarios that lead to mixed traces on cigarette butts. Non-self alleles remain detectable for up to two hours after a kiss on both male and female cigarette butts, revealing a secondary transfer two-hour window that could be crucial in certain forensic cases and warrants further in depth investigation.

Analyses conducted after 30 days also highlighted the need to explore largely whether processing times may affect the DNA results. Minimal individual variation was observed, likely due to both the type of smoking device used and the limited number of samples analysed.

Considering all these findings, it becomes necessary to expand the study in order to provide valuable data that can help investigators interpret the activities underlying mixed traces and reconstruct the dynamics of criminal events.

Declaration of generative AI in scientific writing

During the preparation of this work the authors used ChatGPT-4 (developed by OpenAI) in order to assist with English language editing. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

CRediT authorship contribution statement

Denise Gianfreda: Conceptualization, Formal analysis, Investigation, Writing – original draft. **Beatrice Corradini:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Gianmarco Ferri:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Francesca Ferrari:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Ilaria Borciani:** Methodology, Writing – review & editing. **Rossana Cecchi:** Resources, Supervision, Writing – review & editing. **Anna Laura Santunione:** Resources, Supervision, Writing – review & editing.

Funding

No funding was received for this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors express their gratitude to the volunteer partners who made the study possible.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.legalmed.2026.102803.

Data availability

The data presented in this article are available in the article and from the corresponding author on reasonable request.

References

[1] P. Gill, DNA evidence and miscarriages of justice, Forensic. Sci. Int. 294 (2019) e1–e3, https://doi.org/10.1016/j.forsciint.2018.12.003.

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

[3] R.A.H. van Oorschot, B. Szkuta, G.E. Meakin, B. Kokshoorn, M. Goray, DNA transfer in forensic science: A review, Forensic. Sci. Int. Genet. 38 (2019) 140–166, https://doi.org/10.1016/j.fsigen.2018.10.014.

[4] G. Sallows, D. Taylor, R.A.H. van Oorschot, M. Goray, Contacting surfaces are rarely DNA Free: another look at transfer when both surfaces have DNA, Sci. Justice. 65 (3) (2025), https://doi.org/10.1016/j.scijus.2025.101248.

[5] P. Gill, T. Hicks, J.M. Butler, E. Connolly, L. Gusmão, B. Kokshoorn, et al., DNA commission of the International society for forensic genetics: Assessing the value of forensic biological evidence - Guidelines highlighting the importance of propositions: Part I: evaluation of DNA profiling comparisons given (sub-) source propositions, Forensic. Sci. Int. Genet. 36 (2018) 189–202, https://doi.org/10.1016/j.fsigen.2018.07.003.

[6] P. Gill, T. Hicks, J.M. Butler, E. Connolly, L. Gusmão, B. Kokshoorn, et al., DNA commission of the International society for forensic genetics: Assessing the value of forensic biological evidence - Guidelines highlighting the importance of propositions. Part II: Evaluation of biological traces considering activity level propositions, Forensic. Sci. Int. Genet. 44 (2020) 102186, https://doi.org/10.1016/j.fsigen.2019.102186.

[7] J. Buckleton, D. Taylor, P.S. Allen, J. Curran, T. Kalafut, Reporting the value of biological findings given activity level propositions: A discussion of published recommendations, J. Forensic Sci. (2025), https://doi.org/10.1111/1556-4029.70112.

[8] Y. Watanabe, T. Takayama, K. Hirata, S. Yamada, A. Nagai, I. Nakamura, et al., DNA typing from cigarette butts, Leg Med Tokyo Jpn. 5 (Suppl 1) (2003) S177–S179, https://doi.org/10.1016/s1344-6223(02)00104-9.

[9] L. Casey, S. Engen, G. Frank, Quantitative analysis of the DNA distribution on cigarette butt filter paper, J Forensic Sci. 58 (2) (2013) 470–473, https://doi.org/10.1111/1556-4029.12091.

[10] P. Kelly, E. Connolly, The prevalence and persistence of saliva in vehicles, Forensic. Sci. Int. Genet. 53 (2021), https://doi.org/10.1016/j.fsigen.2021.102530.

[11] M.S. Gegar, G.A. Cisneros, J. Cox, M. Richard, K.A. Currie, Saliva-derived secondary DNA transfer on fabric: The impact of varying conditions, Forensic. Sci. Int. Genet. 72 (2024) 103092, https://doi.org/10.1016/j.fsigen.2024.103092.

[12] S.C. Zapico, G. Roca, A spit in time: identification of saliva stains and assessment of total DNA recovery up to 180 days after deposition, Forensic. Sci. Med. Pathol. 20 (2) (2024) 552–559, https://doi.org/10.1007/s12024-023-00691-6.

[13] M. Breathnach, E. Moore, Oral intercourse or secondary transfer? A Bayesian approach of salivary amylase and foreign DNA findings, Forensic. Sci. Int. 229 (1) (2013) 52–59, https://doi.org/10.1016/j.forsciint.2013.03.029.

[14] J. Kenna, M. Smyth, L. McKenna, C. Dockery, S.D. McDermott, The recovery and persistence of salivary DNA on human skin, J. Forensic Sci. 56 (1) (2011) 170–175, https://doi.org/10.1111/j.1556-4029.2010.01520.x.

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

[16] M. Matte, C. Giancola, J. Millman, Addressing the alternate hypothesis: Transfer and persistence of saliva beneath fingernails, Sci. Justice 63 (4) (2023) 468–476, https://doi.org/10.1016/j.scijus.2023.04.012.

[17] S. Banaschak, K. Möller, H. Pfeiffer, Potential DNA mixtures introduced through kissing, Int. J. Legal. Med. 111 (5) (1998) 284–285, https://doi.org/10.1007/s004140050172.

[18] N. Kamodyová, J. Durdiaková, P. Celec, T. Sedláčková, G. Repiská, B. Sviežená, et al., Prevalence and persistence of male DNA identified in mixed saliva samples after intense kissing, Forensic. Sci. Int. Genet. 7 (1) (2013) 124–128, https://doi.org/10.1016/j.fsigen.2012.07.007.

[19] M. Pesaresi, F. Alessandrini, E. Bignozzi, A. Bernini Di Michele, F. Melchionda, R. Gesuita, et al., The survival of the kiss: presence and persistence of salivary male DNA in mixed samples, Genes 16 (2) (2025) 232, https://doi.org/10.3390/genes16020232.

[20] D. Gianfreda, B. Corradini, G. Ferri, F. Ferrari, I. Borciani, R. Cecchi, et al., Experience based efficient approach for DNA-led identification of highly carbonized human remains, Forensic. Sci. Int. 367 (2025), https://doi.org/10.1016/j.forsciint.2024.112359.

[21] H. Haned, J. de Jong, LRmix Studio 2.1.1. User manual, 2016.

[22] C.C.G. Benschop, J. Hoogenboom, P. Hovers, M. Slagter, D. Kruise, R. Parag, et al., DNAxs/DNAStatistX: Development and validation of a software suite for the data management and probabilistic interpretation of DNA profiles, Forensic. Sci. Int. Genet. 42 (2019) 81–89, https://doi.org/10.1016/j.fsigen.2019.06.015.

[23] P. Gill, T. Hicks, B. Kokshoorn, R.A.H. van Oorschot, D. Taylor, W. Parson, Minimum FSI: Genetics requirements for publishing data on DNA transfer and recovery, given activities, Forensic. Sci. Int. Genet. (2025) 103330, https://doi.org/10.1016/j.fsigen.2025.103330.

[24] L. Carrara, T. Hicks, L. Samie, F. Taroni, V. Castella, DNA transfer when using gloves in burglary simulations, Forensic. Sci. Int. Genet. 63 (2023), https://doi.org/10.1016/j.fsigen.2022.102823.

[25] M. Onofri, C. Altomare, S. Severini, F. Tommolini, M. Lancia, L. Carlini, et al., Direct and secondary transfer of touch DNA on a credit card: evidence evaluation given activity level propositions and application of Bayesian networks, Genes. 14 (5) (2023) 996, https://doi.org/10.3390/genes14050996.

[26] C. Fantinato, P. Gill, A.E. Fonneløp, Non-self DNA on the neck: a 24 hours time-course study, Forensic Sci. Int. Genet. 57 (2022) 102661, https://doi.org/10.1016/j.fsigen.2022.102661.

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

[28] G.E. Meakin, E.V. Butcher, R.A.H. van Oorschot, R.M. Morgan, The deposition and persistence of indirectly-transferred DNA on regularly-used knives, Forensic. Sci. Int. Genet. Suppl. Ser. 5 (2015) e498–e500, https://doi.org/10.1016/j.fsigss.2015.09.197.

[29] A.E. Fonneløp, S. Faria, G. Shanthan, P. Gill, Who Packed the drugs? Application of Bayesian networks to address questions of DNA Transfer, persistence, and recovery from plastic bags and tape, Genes. 13 (1) (2022) 18, https://doi.org/10.3390/genes13010018.

[30] A.E. Fonneløp, H. Johannessen, P. Gill, Persistence and secondary transfer of DNA from previous users of equipment, Forensic Sci .Int. Genet. Suppl. Ser. 5 (2015) e191–e192, https://doi.org/10.1016/j.fsigss.2015.09.077.

[31] A. Gosch, C. Courts, On DNA transfer: The lack and difficulty of systematic research and how to do it better, Forensic. Sci. Int. Genet. 40 (2019) 24–36, https://doi.org/10.1016/j.fsigen.2019.01.012.

[32] B. Kokshoorn, L.H.J. Aarts, R. Ansell, E. Connolly, W. Drotz, A.D. Kloosterman, et al., Sharing data on DNA transfer, persistence, prevalence and recovery: arguments for harmonization and standardization, Forensic. Sci. Int. Genet. 37 (2018) 260–269, https://doi.org/10.1016/j.fsigen.2018.09.006.

[33] P. Gill, A.E. Fonneløp, T. Hicks, S. Xenophontos, M. Cariolou, R. van Oorschot, et al., The ReAct project: Analysis of data from 23 different laboratories to characterise DNA recovery given two sets of activity level propositions, Forensic. Sci. Int. Genet. 76 (2025) 103222, https://doi.org/10.1016/j.fsigen.2025.103222.

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

[35] R.A.H. van Oorschot, G.E. Meakin, B. Kokshoorn, M. Goray, B. Szkuta, DNA transfer in forensic science: recent progress towards meeting challenges, Genes. 12 (11) (2021) 1766, https://doi.org/10.3390/genes12111766.

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

[37] H. Inoue, K. Ono, W. Masuda, Y. Morimoto, T. Tanaka, M. Yokota, et al., Gender difference in unstimulated whole saliva flow rate and salivary gland sizes, Arch Oral Biol. 51 (12) (2006) 1055–1060, https://doi.org/10.1016/j.archoralbio.2006.06.010.

[38] M. Sanga, L. Boiso, H. Lindsten, P. Rådström, R. Ansell, J. Hedman, A panel of PCR-inhibitory reference materials for quality evaluation of multiplex STR analysis kits, Forensic. Sci. Int. Genet. Suppl. Ser. 5 (2015) e317–e319, https://doi.org/10.1016/j.fsigss.2015.09.126.

[39] C.A. Kreader, Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein, Appl. Environ. Microbiol. 62 (3) (1996) 1102–1106, https://doi.org/10.1128/aem.62.3.1102-1106.1996.

[40] P. Gill, Ø. Bleka, A.E. Fonneløp, Limitations of qPCR to estimate DNA quantity: An RFU method to facilitate inter-laboratory comparisons for activity level, and general applicability, Forensic Sci. Int. Genet. 61 (2022) 102777, https://doi.org/10.1016/j.fsigen.2022.102777.

[41] Amurri S, Fazio G, Pirani F, Giorgetti A, Pelotti S, Bini C. DNA recovery and STR profiling from heated tobacco sticks for forensic personal identification. Forensic Sci Med Pathol. 2026 Jan 15. doi: 10.1007/s12024-025-01151-z.