

Comparative analysis of trace DNA samples for STR typing at the crime scene and from material traces in the forensic laboratory

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Abstract

Touch DNA, also known as transfer DNA or trace DNA (tDNA), is becoming an integral part of forensic investigations. tDNA is often the only genetic material recovered from a crime scene, as biological material is often missing. The analysis of tDNA is complicated by several major issues that need to be addressed: low quantity, poor quality of the molecule, high risk of contamination, and mixing of DNA profiles. Evidence obtained from tDNA analysis is often considered unreliable, so the forensic protocol should address and overcome the main problems and maximise the usability of the evidence in legal proceedings. This study evaluates the effectiveness of crime scene samples (CSS) and forensic laboratory samples (FLSs) in short tandem repeat (STR) typing using Fisher's exact test. CSS were collected at the crime scene by swabbing sites and objects, while FLSs were obtained by a forensic geneticist under laboratory conditions from material traces seized at the crime scene. The samples were taken from actual cases investigated by the police of the Czech Republic. The results, analysed using the R software, showed that 56.2% of the tDNA samples could provide STR profiling results of the same quality as samples with proven presence of biological material (semen, saliva and blood). The study demonstrated that tDNA sampling is practical and that DNA profiles generated from tDNA samples have the potential to identify persons at the individual identification level. The study highlighted the need for refinement and development of new techniques to determine the amount of biological tDNA material in the traces of the crime scene and also in the examination of the material traces in the forensic laboratory, as this direction is desirable given the number of traces examined compared to the other biological materials mentioned above.

Keywords: forensic sciences; forensic genetics; touch DNA; transfer DNA; trace DNA; tDNA; tDNA sampling; DNA profiling; STR profiling; DNA swabbing; fingerprint

Introduction

Touch DNA, also known as trace DNA, transfer DNA, or tamper DNA (generally tDNA), is defined as DNA from cells that does not originate from biologically defined traces (e.g., blood, semen, saliva) and remains on the surface of an object when manipulated by a suspect [1]. Samples of tDNA are characterised by low concentrations of DNA as well as degradation, often a mixture of DNA from several different donors. Transmission of tDNA occurs primarily through contact between the donor and the object (trace) [2–6], or secondarily through [2] contact with a contaminated object [6–9]. Additionally, Helmus et al. [10] described tertiary transmission.

Factors influencing tDNA maintenance and adhesion are external [11] tissue type, cell type, free DNA content, mode of transfer, conditions and duration of deposition [9, 12], environmental conditions (heat, UV, humidity, duration of deposition) [7, 9, 13, 14], surface properties, surface type [15], porosity [16, 17], adhesion, electrostatic forces, presence of

microorganisms and inhibitors [18], contact area [5], previous contacts [4, 6], etc. Internal factors include donor age and sex [10, 19, 20], physical and health status [21–23], and hygiene habits [22, 24].

Advanced molecular biology methods and biostatistical approaches in forensic science enable tDNA analysis to be used as evidence. To obtain high-quality results that can be clearly interpreted, it is essential to have a detailed knowledge of tDNA transfer and preservation processes and to optimise laboratory methods and procedures. Forensic experts are particularly focused on testing and optimising tDNA sampling (swabbing, use of tapes, etc.) and extraction methods, with limited attention paid to the timing of tDNA sampling [25]. The most sophisticated techniques, methodologies, and approaches to genetic analysis in the forensic laboratory cannot compensate for the insufficient amount or poor quality of DNA recovered from the traces on the tDNA swab.

Crime scene evidence, including tDNA swabs, is collected primarily from the crime scene or from items recovered as physical evidence from the crime scene. The biological

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material in physical evidence is secured in the forensic laboratory after transport to the workplace. Depending on where the tDNA swabs were collected, either directly at the crime scene or later in the forensic laboratory, the evidence can be divided into two groups. The swabs collected by the forensic technician at the crime scene are referred to as crime scene samples (CSS). The tDNA swabs taken from the evidence transported to the forensic laboratory were collected by the forensic scientist and are referred to as forensic laboratory samples (FLS).

The location of the tDNA swab depends on the situation and condition of the crime scene. The general rules established by tDNA swab research are applicable here and are also based on the practice and experience of the forensic technician. In general, these are places and objects that the perpetrator was likely to have been touched, potentially transferring their biological material in the form of tDNA to the swab site. Specifically, the seizure of biological material is required when such sites and objects cannot be secured in their entirety and transported to the forensic laboratory. The forensic technician selects the swab sites after obtaining information from the crime reporter, who knows the condition of the crime scene prior to the crime and is therefore able to indicate the changes that have occurred and where the perpetrator's biological material could potentially be located. Samples of tDNA taken from these areas and large objects (interior furnishings) are called CSS.

Items associated with the commission of a crime and recovered as a whole from the crime scene are referred to as physical evidence. These can be objects that the offender may have touched (cups, broken pieces, parts of objects separated from larger units by the offender's actions) or objects that the offender brought to the scene and left there. Again, the information from the informant is needed to make a decision about the seizure of physical evidence. The physical evidence is then taken to the forensic science laboratory with the relevant documentation, where it is examined and a swab is taken from the relevant areas and marked as FLS. A relevant area is the one that the perpetrator may have touched during handling and may have left their biological material on the object.

The main content of the study is to compare the success rate of swab samples taken directly from the crime scene with samples taken from physical traces in a forensic laboratory. Although it is generally known that tDNA traces are not as successful in DNA profiling as blood and semen traces, according to available statistics conducted by one of the authors, more than 68% of tDNA traces were submitted for examination during the study period from 2017 to 2020. The results of the study should show which samples are less successful in genetic analysis, formulate recommendations and update the standard operating procedure for tDNA sampling. The increase in success rates, which will be reflected in the creation of a suitable DNA profile for comparison, will contribute to more effective police intervention in identifying offenders and reducing crime.

Materials and methods

Biological material

During the investigation, biological samples were taken for analysis by a criminalistic technician and a criminalistic

expert from the Laboratory of Forensic Biology and Genetics of the Department of Criminalistic Technology and Expertise in Brno, Regional Directorate of the South Moravian Region, Police of the Czech Republic. The samples were taken in the period June 2017–July 2020 in the regions of the Czech Republic—South Moravia, Vysočina and Zlín. A total of 1 343 samples were included in the study. Forensic traces are unique and cannot be repeated or sampled.

Throughout the study, maximum uniformity of sample analysis was maintained.

Methods used

Collection of tDNA samples

Two different sampling approaches—crucial parameters in result evaluation—were used in this study. Samples were collected directly at the crime scene (CSS) and also swabbed in laboratories during the examination of objects at the crime scene (FLS). A sample was classified as USABLE if DNA was detected using a real-time quantitative PCR (qPCR) strategy. If no DNA was detected in the sample after quantification, the sample was further concentrated using a vacuum centrifugal concentrator (Eppendorf Vacufuge Plus Basic Device; Eppendorf AG, Hamburg, Germany). The isolated samples treated in this way were quantified again using the same method. Samples in which no DNA was detected even after evaporation were classified as UNUSABLE.

The samples were divided into two groups according to the sampling method. In Group A, CSS were collected, as swabs at the crime scene during the investigation. tDNA was extracted from smooth surfaces and objects at the crime scene by trained forensic technicians. Swabs were taken from incomplete dactyloscopic evidence, particularly fingerprints. Samples were taken from surfaces of objects and objects that could not be transported to the Office of Criminalistic Technology and Expertise (OKTE) in Brno. DNA analysis of tDNA samples was carried out as a matter of priority, at the latest 4 weeks after collection. In Group B, FLS sampling was performed under controlled conditions in forensic laboratories. Small items such as tools, everyday objects, clothing, parts of objects, printed matter, hats, shoes and other items directly related to the crime were sampled throughout the study. The items were thoroughly examined and one or more surface swabs were taken. Particular attention was paid to folds, edges, handles, seams, crevices and similar details with a higher risk of adherence of biological material. The average interval between field investigation and laboratory collection of tDNA samples was 1 to 6 months. The list of samples is presented in Table 1, including key sample characteristics.

Each trace for DNA analysis was registered and numbered in the forensic laboratory according to current standard operating procedures (SOPs), which were in line with international guidelines [26]. Police officers and forensic technicians received regular training in the process of providing traces for biological analysis every 3 years at the Police Training Centre and further training from OKTE staff, where the methodology of sample collection was standardised. Minor differences in sampling were regional, and technicians trained in other departments of forensic technology and expertise used the same sampling methodology.

Group A samples were collected using 4N6FLOQSwabs® (Copan Diagnostics Inc., Murrieta, CA, USA). The swabs

Table 1. Summary of the number of cases with tDNA and the number of trace samples seized.

Year	Number of cases		Number of samples		
	Total	Cases with tDNA	CSS	FLS	Total
2017	83	51	65	41	106
2018	215	177	178	208	386
2019	332	214	358	183	541
2020	171	135	209	101	310
Total	801	547	810	533	1 343

CSS: crime scene samples; FLS: forensic laboratory samples.

containing the biological sample were collected immediately after collection/swabbing, securely in an Eppendorf tube or in a plastic container in a plastic bag. The cap of the tube was also perforated. The secured DNA sample was transferred to the genetic analysis laboratory in sterile packaging. DNA samples were stored in a dark, dry room at a stable room temperature until analysis. All substances/kits used for isolation, quantification and amplification were for forensic

use only and were tested prior to use. The sample processing procedure is shown in Figure 1.

DNA isolation and quantification

DNA was isolated using the QIAamp® DNA Blood Kit [27] or the QIAamp® Investigator® Kit [28] (Qiagen, Hilden, Germany) according to the manufacturer’s recommendations. The final volume of the DNA extract was 100 µL.

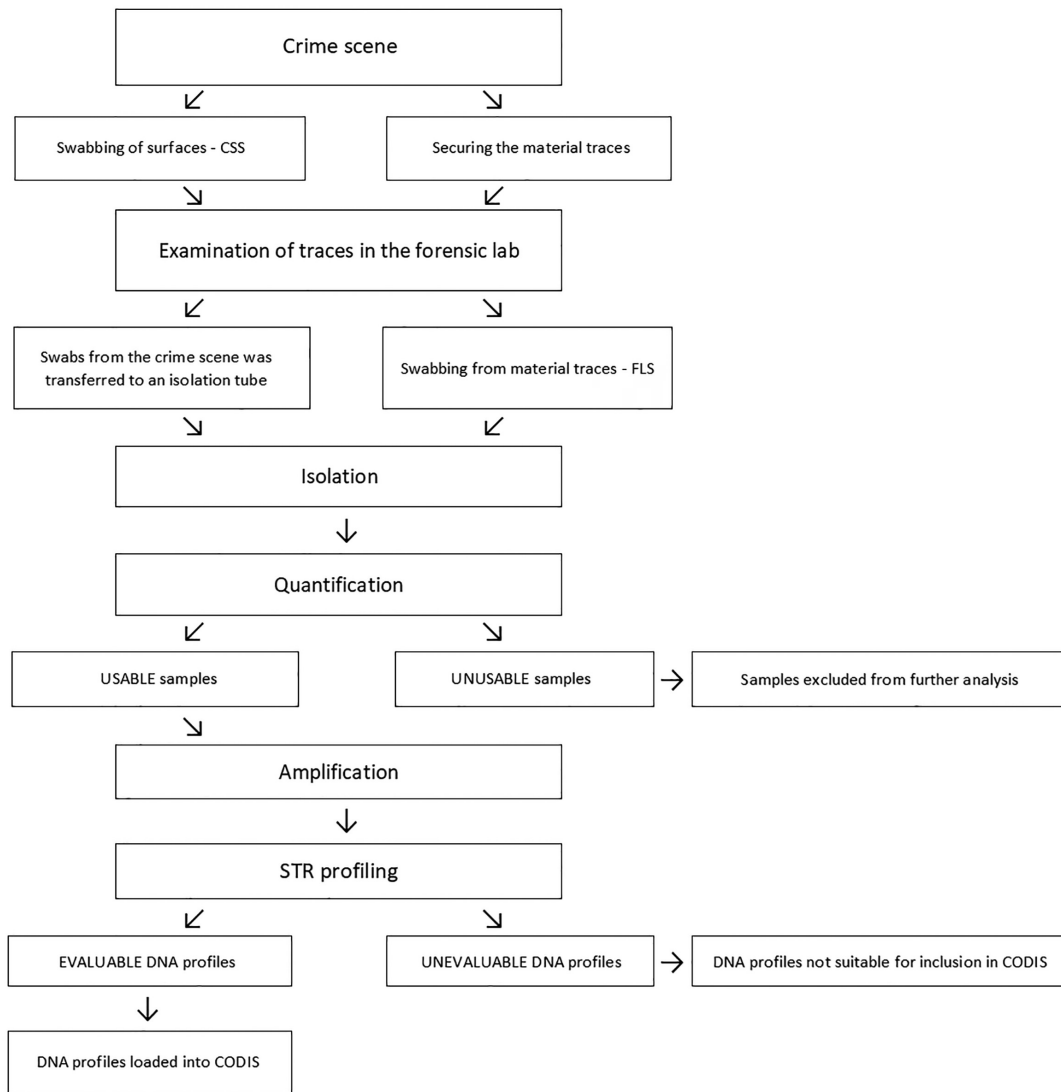


Figure 1 Sample processing from crime scene examination to insertion of STR analysis evaluation. CSS: crime scene sample; FLS: forensic laboratory sample; STR: short tandem repeat; CODIS: Combined DNA Index System.

The isolation of tDNA was followed by real-time PCR quantification. Two kits were used according to the manufacturer's instructions: the PowerQuant® system (Promega, Madison, WI, USA) on a 7500 real-time PCR system (Applied Biosystems™; Thermo Fisher Scientific, Foster City, CA, USA) and the Investigator Quantiplex® kit (Qiagen) on a Rotor-Gene® real-time cyclor (Qiagen), following the manufacturer's recommendations. The cut-off point for sample exclusion from further analysis was established at 0.002 5 ng/μL for samples analysed on the Real-Time 7500 and 0.004 ng/μL for samples analysed on the Rotor-Gene® instrument, respectively, in a validation study carried out by laboratory staff. All samples were run in duplicate. Samples with concentrations below 0.01 ng/μL tDNA were concentrated by vacuum evaporation using the Concentrator plus 5305 (Eppendorf) for 15 min at 45 °C. The final volume of the sample was halved, i.e., approximately (50 ± 5) μL (10%), and was quantified again. The sample was retained in the study after the effective concentration and classified as USABLE. Samples below the DNA detection limit were excluded from further analysis and considered insufficient for DNA profiling according to the current DNA profiling methodology. These samples were considered UNUSABLE.

STR profiling

DNA profiles were generated using PowerPlex® ESI 17 [29], PowerPlex® ESX 17 [30] and/or PowerPlex® Fusion [31] kits (Promega) according to the manufacturer's recommendations. The maximum recommended template volume was used for amplification, unless the quantification results indicated otherwise.

Fragmentation analysis was performed on the Applied Biosystems Genetic Analyser (GA) 3130 and 3500 [32], according to the manufacturer's recommendations. STR profiles were evaluated using GeneMapper™ version 3.2 software [33] or GeneMapper™ ID-X software [34]. The minimum peak height was 50 relative fluorescence units (RFU) for GA 3130 and 80 RFU for GA 3500.

DNA profiles were evaluated according to the applicable criteria of the laboratory SOP (ISO/IEC 17025, General Requirements for the Competence of Testing and Calibration Laboratories) [35], which was in line with European and global guidelines for evaluating DNA profiles [36–39].

The results of the STR profiles and fragmentation analysis were divided into two groups: an interpretable DNA profile, suitable for comparison at the level of individual identification, and an uninterpretable DNA profile, which was excluded from further evaluation as UNUSABLE. Based on the results of the fragmentation analysis, DNA samples were classified as EVALUABLE and UNEVALUABLE. An EVALUABLE DNA sample provided a complete DNA profile of an individual or an incomplete DNA profile of an individual whose random match probability (RMP) was less than 10–12 and a mixed DNA profile with one or two major contributors in the DNA mixture. An UNEVALUABLE sample was a DNA profile that is incomplete and had an RMP value greater than 10–12, incomplete DNA mixtures with visible dropout or other stochastic effects, and indistinguishable mixtures with different numbers of contributors [40].

When evaluating the DNA profile, the above parameters were taken into account, and the overall appearance of the DNA profile was also assessed, such as peak height, number

of peaks at each locus and homogeneity of results when the DNA analysis was repeated. This was often the case when (1) a clear distinction between major and minor components was required, (2) reference material was not available, (3) it was not possible to determine the “owner” of individual alleles, (4) RFU height corresponded to the rate of DNA degradation, (5) RFU values below 200 indicate that the presence of alleles in the DNA profile is uncertain, or (6) repeated amplification and subsequent fragmentation analysis led to a change in peak height and also to their disappearance. Any doubt about the identified allele that would cast doubt on the unambiguousness of the conclusion of the genetic analysis must be expressed by statistical calculation. If the conclusion was clear, it was advisable to support it by repeating the fragmentation analysis and checking the consistency of the results. In the Czech judicial system, in cases of doubt, the *in dubio pro reo* principle is always applied.

Statistical analysis

The differences between the CSS and FLS groups in usability (sufficient DNA) and evaluability (DNA profile suitable for comparison) of the samples were tested using Fisher's exact test. Comparisons were always made first for all tDNA samples examined during the study period and included in the study. Then, only samples from those criminal cases where samples were collected simultaneously by both methods (CSS and FLS) were selected. The differences between CSS and FLS in the usability and evaluability of samples from these cases were then tested separately. Differences were tested at different levels of significance ($P < 0.0001$, $P < 0.01$ and $P = 0.5$, with the specific P -values always given). All statistical calculations were performed in R software (version R-4.1.3; www.r-project.org), a language and environment for statistical computing and graphics [41], which was originally written by Robert Gentleman and Ross Ihaka—also known as “R&R”—from the Department of Statistics at the University of Auckland.

Results

The present study is based on real data from the Police of the Czech Republic, data and results of investigations of criminal cases in the regions of South Moravia, Zlín and Vysočina. The criminal traces were sent for examination to the Department of Criminalistics and Expertise in Brno, so this is not a controlled experiment in a laboratory environment. The results can be summarised and described simply by categorising them on the basis of the results of tDNA quantification of the samples and subsequent STR profiling. The statistical comparison is described below.

The same parameters were established for all samples with regard to the method of isolation, quantification and the established thresholds for the minimum amount of DNA, and amplification and evaluation of the STR DNA profiles. The system remained unchanged for 3 years, thus maintaining consistency in the way the traces/samples were processed and the subsequent evaluation of the results, and therefore the study was limited to 3 years, with only one expert performing the study and evaluating the samples.

The study included a total of 801 criminal cases in which 1 343 tDNA samples were examined, representing 68.28%

(converted to number of criminal cases) the total number of samples examined by an expert in the laboratory during the study period (Table 1).

The quantity and quality of DNA in the samples were assessed at two points during the DNA analysis, with samples that did not meet the criteria discarded. The first point was the evaluation of the quantification results and the second was the evaluation of the STR profiling results. First, the results after quantification were compared and samples with sub-quantitative amounts of DNA in the isolate were marked as UNUSABLE samples. Samples with sufficient amounts of DNA were marked as USABLE. Table 2 first shows the results for each type of CSS and FLS. Of the CSS category, i.e., samples collected by the forensic scientist at the crime scene, samples with sufficient amounts of DNA for profiling were found in 445 cases (54.9%); of the FLS, where the forensic geneticist collected tDNA during the laboratory examination, samples with sufficient amounts of DNA were found in 356 cases (66.8%). FLS were more likely to be usable despite the significant delay between the seizure of evidence in the field and the collection of tDNA samples in the laboratory. This delay averaged between 1 and 6 months, during which time tDNA degradation may have occurred. The difference in sample availability between the CSS and FLS groups was highly statistically significant ($P < 0.0001$), indicating that a greater amount of DNA was recovered from the physical traces.

The difference in availability between the CSS and FLS strategies was even greater when comparing samples from criminal cases where both crime scene swabs (CSS) and forensic laboratory fingerprints (FLS) were collected. In Table 3, 47.8% of CSS were found to have sufficient DNA compared to 70.5% of FLS; the difference was found to be highly statistically significant ($P < 0.0001$). Again, the CSS appeared to contain a lower amount of DNA compared to the physical evidence swabs.

The quantification results of the samples marked USABLE in Tables 2 and 3 are summarised in Figure 2, as the amount of DNA measured was above the limit of elimination. The FLS samples contained sufficient DNA in several cases, regardless of whether all samples were compared with each other or whether only crime cases were included in the evaluation where some samples were secured by the technician (CSS) and some were swabbed in the laboratory (FLS).

In practice, it appears that the higher concentration of DNA in FLS is usually due to the fact that the swab is taken from the material trace found at the crime scene, on the assumption that the offender handled the object at the crime scene or that the object belonged to the offender according to the established facts. Therefore, there is a higher probability that the offender used the object for some time and a higher amount of DNA was transferred to the surface of the object or tool, which was confirmed by the results.

The low concentration of DNA in the tDNA swabs, which was judged to be insufficient, is due to the offender's behaviour at the crime scene, which in some cases can be shown by closed-circuit television footage of the crime scene, where the offender's movements are recorded and it is evident that they touches the places and objects only very briefly, or has put on gloves before the burglary. It also depends on whether the perpetrator is a good or bad shyster, for whom even prolonged and intensive contact would not guarantee the transfer of sufficient quantities of biological material.

Higher concentrations of DNA were measured in samples obtained by forensic fingerprint swabbing with tDNA carried out in the forensic laboratory after examination by forensic dactyloscopy, which visualises fingerprints. A tDNA swab was taken from the site of the visualised dactyloscopic print. The same procedure can be followed at the crime scene in cases where a forensic technician visualises a dactyloscopic print that is not suitable for examination by forensic dactyloscopy. However, a trace likely to have been left by the perpetrator at the scene may be useful for forensic genetic examination and is preserved in the form of a tDNA swab. This swab is stained with dactyloscopic powder and can be distinguished from other swabs.

The second point at which the results of the DNA analysis were evaluated was the evaluation of the DNA profile. The DNA profiles were divided into two groups, labelled EVALUABLE and UNEVALUABLE, respectively. The samples labelled EVALUABLE were used to determine DNA profiles suitable for comparison, while the UNEVALUABLE group consisted of samples that yielded DNA profiles unsuitable for comparison.

An EVALUABLE DNA sample (STR profile) was obtained in 351 cases out of a total of 801 EVALUABLE samples (43.8%). As shown in Table 4, for the CSS strategy, an EVALUABLE DNA sample was detected in 173 DNA extracts, i.e.,

Table 2. Usability of the samples for all criminal cases.

Group	USABLE samples (n, %)	UNUSABLE samples (n, %)	Total (N)
CSS	445 (54.9)	365 (45.1)	810
FLS	356 (66.8)	177 (33.2)	533

CSS: crime scene samples; FLS: forensic laboratory samples.

Table 3. Usability of the samples for the criminal cases where both CSS and FLS were performed.

Group	USABLE samples (n, %)	UNUSABLE samples (n, %)	Total (N)
CSS	65 (47.8)	71 (52.2)	136
FLS	74 (70.5)	31 (29.5)	105

CSS: crime scene samples; FLS: forensic laboratory samples.

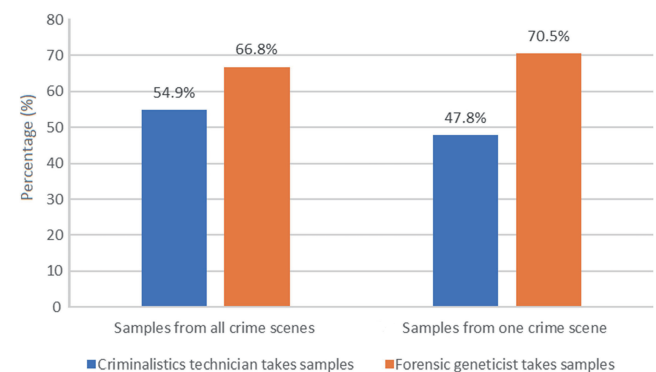


Figure 2 Usability of the samples for the criminal cases.

38.9% of the 445 USABLE samples obtained using the CSS strategy. In the case of the FLS strategy, the sample with the EVALUABLE DNA profile result was detected in 178 DNA isolates, i.e., 50% of the 356 USABLE samples obtained using the FLS strategy. The FLS strategy produced statistically significantly more EVALUABLE results than the CSS strategy ($P < 0.01$).

Interestingly, the difference in the number of EVALUABLE samples was much smaller and not statistically significant when comparing samples from criminal cases where both swab samples were collected from the crime scene and physical evidence was collected from the crime scene and later tested in the laboratory. Table 5 shows the samples recovered using the CSS and FLS strategies. The DNA profiles obtained from the samples in the CSS group were suitable for comparison in 50.8% of cases compared with 56.8% in the FLS group ($P = 0.50$).

In percentage terms, more CSS and FLS were discarded than after quantification. DNA profiles that were incomplete, biological material from multiple individuals in proportions where individual contributors could not be distinguished, or a combination of both situations were discarded. The obtained results indicate that although sufficient DNA was detected in the sample for DNA profiling, this does not automatically imply sufficient DNA quality and a positive contribution of the trace to the criminal case.

The results of STR profiling of the samples considered suitable for comparison - EVALUABLE are summarised in Figure 3. The differences in the number of positive samples were smaller, but it was still observed that the samples recovered from the factual traces in the forensic laboratory (FLS) provided DNA profiles suitable for comparison.

The FLS strategy was always found to be more advantageous, and the samples contained more DNA of higher quality and were more useful for the criminal case than the samples seized at the crime scene, i.e., the CSS strategy.

Discussion

Although tDNA analysis is commonly perceived to be new in forensic genetics, such claim is actually not true. The first fingerprint DNA analysis was performed 25 years ago [42]. With the development of molecular biological methods, tDNA analysis has become an integral part of forensic investigation.

Although tDNA is usually present in low concentrations and is therefore classified as a “low copy number” sample, it is possible to determine not only the STR profile but also the phenotypic characteristics of the donor.

Most published tDNA studies address one of the five key questions:

- 1) What is the origin of the tDNA [22, 43, 44]?
- 2) What are the transmission and conservation patterns [39, 45, 46]?
- 3) What are the factors that influence the conservation of tDNA [17, 47, 48]?
- 4) What forensic genetic techniques and methods are most appropriate for their analysis, from swabbing to evaluating the results [18, 20, 49]?
- 5) What is the success rate of tDNA sampling in forensic practice [50–52]?

The results and conclusions of the publications listed in Questions 1–4 were used to develop a mechanism for securing and processing forensic tDNA traces. This study focused on answering Question 5. tDNA is not one of the traces with a high success rate in DNA analysis, where success is judged by the result of STR profiling, i.e., the DNA profile suitable for comparison with that of a person. tDNA samples are the type of trace most frequently submitted for examination in our laboratory (on average, they account for more than 68% of all requests for DNA analysis, more than 72% of all traces examined). By using data and knowledge of the mechanism of trace detection, standardised packaging protocols for sample transportation, and the analytical procedures for DNA analysis of tDNA samples, we obtained the results presented in Tables 2–5 and summarised in Figures 2 and 3.

Securing tDNA traces is an urgent and non-repeatable task, even considering the expected small amount of biological material contained in the trace. Therefore, the quality and quantity of DNA in the trace to be secured is critical. The crime scene, even in the broadest sense, is the only source of forensic evidence. The aim of forensic trace analysis is to identify the perpetrator of a crime, and forensic DNA analysis, like forensic dactyloscopy, allows the comparison of a forensic trace with a comparison sample to be evaluated at the level of individual identification. Thus, successful DNA analysis can be defined as the establishment of a DNA profile suitable for comparing with a comparison sample. The forensic traces were divided into two groups according to the location of the seizure. The forensic technician collected the tDNA swabs

Table 4. Evaluability of the samples for all criminal cases.

Group	EVALUABLE samples (n, %)	UNEVALUABLE samples (n, %)	Total (N)
CSS	173 (38.9)	272 (61.1)	445
FLS	178 (50.0)	178 (50.0)	356

CSS: crime scene sample; FLS: forensic laboratory sample.

Table 5. Evaluability of the samples for the criminal cases where both CSS and FLS were performed.

Group	EVALUABLE samples (n, %)	UNEVALUABLE samples (n, %)	Total (N)
CSS	33 (50.8)	32 (49.2)	65
FLS	42 (56.8)	32 (43.2)	74

CSS: crime scene sample; FLS: forensic laboratory sample.

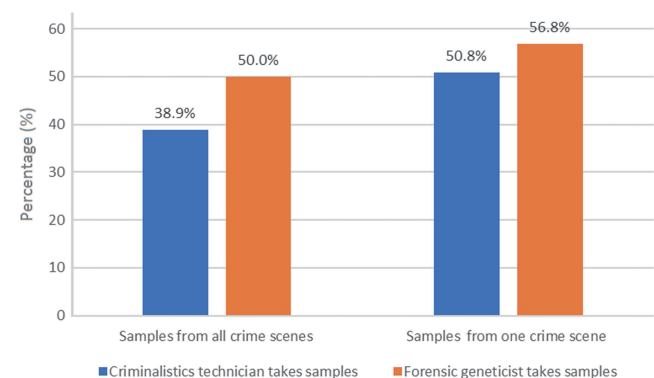


Figure 3 Evaluability of the samples for the criminal cases.

at the scene of the crime using the available means, without using any method to visualise the tDNA. The forensic geneticist to whom the physical evidence was submitted followed the same procedure. The swabs were taken without testing for the presence of biological material. In this case, where the degree of degradation is a possible parameter to be monitored in addition to the concentration itself, only quantification can determine whether the tDNA is present and if so, in what quantity and quality. It is therefore appropriate to determine the usefulness of criminal traces from a police investigation point of view at the beginning of DNA analysis, or to rationalise the selection of traces.

When comparing the results observed at the two checkpoints (quantification results and determination of a comparable DNA profile), a higher success rate was always found for samples seized using the FLS strategy, which is consistent with the results of publications that analyse traces seized from objects and items that have been shown to have been touched by the person of interest [5, 50, 51]. The lower success rate of the CSS shown in Figure 2, which is based on the results in Tables 2 and 3, may be influenced by the fact that it cannot be ruled out with certainty that the perpetrator used protective equipment such as gloves. Limited knowledge of the perpetrator's behaviour and other internal factors may bias the conclusions drawn from the results of the analyses performed and evaluated. We return to the need to test for the presence of DNA in the trace at the scene and also in the laboratory prior to swabbing.

More than 70% of the swab samples recovered by the forensic laboratory contained sufficient DNA for profiling, and almost 57% of the DNA profiles obtained from these samples were suitable for comparison with the person's DNA profile (Table 5). However, the percentage success rate in terms of the number of samples collected by the forensic scientist at the crime scene that were suitable for comparison with the DNA profile was 50.8% (Table 4). A lower percentage of DNA profiles suitable for comparison was also found when comparing pure samples seized by the CSS and FLS strategies (Table 4). The comparison of the results of the determination of USABLE DNA profiles, divided into Tables 4 and 5, is summarised in Figure 3.

The difference between the DNA content of CSS and FLS was highly significant, almost 12% (all samples) and 23% (within one case), respectively, which may be somewhat surprising. The difference in DNA profile quality was again 12% (all samples) and 6% (within one case). It would seem that if DNA is found in both the crime scene and laboratory isolates within a single case, it can be concluded that the perpetrator was demonstrably present at the crime scene and left his DNA on the objects rather than at the sites where the forensic technician took the swabs. To increase the efficiency of tDNA sampling at the crime scene and in the forensic laboratory, it is necessary to introduce a method that allows visualisation of human cells at the site of the presumed presence of DNA, without the loss of biological material and the degradation of quality that would affect the determination of the DNA profile. The staining of human DNA with Diamond™ Nucleic Acid Dye [53] appears to be effective. The implementation procedure was conducted in two phases.

Phase 1: Testing tracks prepared under laboratory conditions mimicking the mechanism of tDNA track formation by primary, secondary and tertiary transfer using Diamond™ Nucleic Acid dye. The functionality is also tested on the

surfaces of material traces studied in forensic dactyloscopy, partially consumed food, and other problem areas. The results of DNA analysis of artificially created forensic traces are compared with those of previously published work and, if necessary, modify the procedure for the type of trace/surface. This initial testing should facilitate the introduction of the method into police practice.

Phase 2: Introduction of the method into police practice in the selected area. Criminal traces with a proven human tDNA content are secured from the tested surfaces and objects at the crime scene, and subjected to genetic analysis, i.e., DNA isolation and quantification and DNA profiling. Over time, the results of the recovered and analysed samples are compared with those of samples recovered without human tDNA testing.

The conditions and methods used in the tests should be similar so that the data obtained can be compared without bias. The detection of human DNA on the surface of material traces should be implemented in practice in the forensic laboratory when tDNA is secured.

Conclusion

This article presents real data from forensic cases in which tDNA samples, commonly submitted for processing in the field of forensic genetics, were submitted for examination. On the basis of published results, the submission of tDNA samples has been shown to be useful because it provides data that allow the identification of traces at an individual level, just like blood or semen traces. These results show that the problem with DNA profiling in terms of DNA quality and quantity is not in the DNA analysis method itself, but in the mechanism of finding traces with tDNA material and the associated decision as to whether it is a relevant trace suitable for seizure and subsequent investigation. Therefore, a method that does not affect the quality and quantity of DNA in the trace by testing and, ideally, reliably detects only human biological material. This would be of benefit not only at the crime scene but also in the forensic laboratory examination of material traces collected at the crime scene without testing, on the basis of available information and the assumption that the perpetrator touched or brought them to the crime scene and left them there. Examination of tDNA traces would be more efficient, not only less costly, but also more beneficial to the criminal process by increasing the number of DNA profiles available for comparison. The authors' method of staining human cells with Diamond™ Nucleic Acid dye, which binds to the DNA contained in the cells without adversely affecting subsequent DNA analysis and DNA profiling, appears promising.

Authors' contributions

Hana Svobodová conceptualized the study, collected and analyzed data from criminal trace examinations conducted at OKTE Brno, drafted the manuscript, and revised it based on reviewers' feedback. Kristýna Brzobohatá contributed to the Introduction and Discussion sections, revised methodological components, and consulted on data evaluation strategies. Tomáš Zeman and Radek Mitáček jointly advised on data evaluation, developed statistical methods, performed statistical analyses, and prepared Tables 2–5. Eva Drozdová provided consultation and reviewed the manuscript prior to

submission. All authors participated in the final revision and approved the final version of the manuscript.

Compliance with ethical standards

The Ethics Committee of the Masaryk University waived ethical review for this manuscript. This study utilized anonymized crime scene evidence containing biological material. As the individuals from whom this material originated could not be identified, obtaining informed consent was not feasible. The South Moravia Regional Police authorized the use of the data and approved its publication.

Disclosure statement

The data originate from actual criminal cases handled by the Criminalistics and Expertise Department of the South Moravia Regional Police, with crime scene sampling performed in accordance with legal standards and ISO/IEC 17025:2017-accredited laboratory protocols (maintained since 2008).

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