REVIEW

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Trace DNA: A Review, Discussion of Theory, and Application of the Transfer of Trace Quantities of DNA Through Skin Contact

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ABSTRACT: Advances in STR PCR DNA profiling technology allow for the analysis of minute quantities of DNA. It is frequently possible to obtain successful DNA results from cellular material transferred from the skin of an individual who has simply touched an object. Handling objects, such as weapons or other items associated with a crime, touching surfaces, or wearing clothing, may represent sufficient contact to transfer small numbers of DNA bearing cells, or trace DNA, which can be successfully analyzed. With this minimal amount of contact required to yield a suspect profile comes tremendous crime solving potential, and a number of considerations for prudent application, and the maximization of evidentiary value. Evidentiary materials not previously considered must be recognized and preserved, and the resulting DNA type profiles interpreted in their proper forensic context.

KEYWORDS: forensic science, DNA typing, polymerase chain reaction, trace DNA, contact DNA, LCN DNA, short tandem repeat, skin, contact, slougher, fingerprints

Individualization of human beings has long been a challenge facing law enforcement. An ideal system includes identifying characteristics unique to each individual, with features that do not change over time, which can be catalogued such that suspect samples can be compared against a set of known reference samples. Taken in the context of modern forensic science, these features should be inherent within whatever evidence would be left at the scene of a crime, to unambiguously link the perpetrator to the crime. At one time this ideal appeared to be embodied by fingerprint comparison. This may have originally been the case; that is until the criminal population became aware of the limitations of fingerprints, and began wearing gloves to the scenes of crime. The best evidence is that which is recognized by law enforcement officials, yet not by individuals perpetrating the crime. If suspects did not know about fingerprints, would they wear gloves to a crime scene? Trace DNA represents that potential.

In 1985, Jeffreys et al. (1,2) first used restriction fragment length polymorphisms (RFLPs) to exclude a wrongfully accused suspect in the now famous Colin Pitchfork murder case, and then to provide an invaluable investigative lead, which proved instrumental in solving the case. The resulting "DNA fingerprints" still carried conventional forensic serology's ability to definitively eliminate suspects (3), however precipitated a revolution in the area of forensic association. Far less questioned biological material was required to produce profiles for comparison, and DNA was demonstrated to be far more stable than the proteins relied upon in forensic serology. Using RFLP technology, sample sizes of approximately 250 ng were targeted to produce DNA profiles. Usable DNA profiles could be produced with as little as 30 ng of high molecular weight (undegraded) DNA template.

Short Tandem Repeats (STRs) have subsequently replaced RFLPs as the nuclear DNA polymorphism of choice for forensic comparisons. While the level of polymorphism per locus may be less, the increased number of loci examined provides sufficient variation for discrimination between individuals (4). The smaller fragment size required by STRs permits a greater likelihood of successfully obtaining DNA profiles with samples containing degraded DNA (5). Through the incorporation of fluorescence detection, and internal lane standards, analyses of PCR loci could be multiplexed and alleles sized (6,7). Thus, great discrimination could be achieved, with a further decrease in sample size required to generate a complete DNA profile. Early generation multiplex PCR STR profiling used in the early to mid 1990s targeted between 1 and 20 ng of purified DNA for full profile development.

Use of the PCR technique has enabled DNA analysis on very minute and badly degraded biological samples, and on a number of exhibit types not previously considered for differentiation at the genetic level. Single human hairs were successfully compared by Higuchi et al. (8) in 1988, adding a new genetic dimension to traditional microscopic comparison of hairs. Buccal cells on cigarette butts were analysed by Hochmeister el al. (9) in 1991. Ancient skeletal remains were processed by Hagelberg and Sykes (10) in 1989, stimulating the field of forensic anthropology. PCR DNA typing has been used to identify extensively charred partial remains from individuals involved in fires and mass disasters (11,12). Blood, seminal fluid, vaginal fluid, dental pulp, human dandruff, urine, and saliva have also been used as sources of DNA for identification (13–20). Skin cells were targeted as a potential source of DNA, as van Oorschot and Jones (21) obtained DNA from swabs of hands, and handled objects. Transfer of skin cells during stran-

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gulation was examined by Wiegand and Klieber (22). A murder case involving suspect DNA typing profiles recovered from the handles of knives was reported by Wickenheiser and Challoner (23,24). Ever increasing sensitivity of STR PCR DNA profiling continued to expand the scope and variety of exhibits, which could be successfully analyzed.

Development of "megaplex" PCR STR systems, simultaneously examining anywhere from 9 to 15 loci simultaneously, have reduced the target DNA requirement to 1 ng of purified DNA (PE Applied Biosystems, Perkin-Elmer, Foster City, CA, and Promega Corp., Madison WI). This further reduction in sample size required for successful DNA profile development has fuelled an explosion in the variety of successfully processed exhibits. Use of epithelial cells represents a particularly novel and useful dimension of this increased potential. Casework examples from the Royal Canadian Mounted Police (RCMP) Forensic Laboratory in Regina, Saskatchewan include the following evidentiary items from the following crime scenes (25–27, Table 1):

1. The nose and ear pieces of glasses dropped at a crime scene.
2. Both the adhesive and backing side of adhesive tape used in the construction of a crude home made club used in an armed robbery.
3. Band-Aids used to protect against fingerprint deposition at a crime scene.
4. Handles of plastic shopping bags, screwdrivers, knives, and a variety of weapons.
5. Ligatures used in strangulation, including electrical cord, rope, and twine.
6. A single smudged fingerprint on the door pull on an exterior door at the scene of a murder.
7. Shoe laces from running shoes left at a crime scene.
8. The doorbell from a home invasion case, which included a mixture from a suspect, and a home care worker who visited the complainant the previous day.

TABLE 1—*Unusual exhibit material yielding successful DNA profiles using PCR STR typing. Source: RCMP Forensic Laboratory Services (25).*

DNA Source	Exhibit Type	
Hands	Arm-rest (automobile)	Hash-like ball (1 cm diameter & hand-rolled)
	Baseball cap (brim)	Hold-up note
	Binder twine	Ignition switch
	Bottle cap	Keys
	Chocolate bar (handled end)	Knife handles
	Cigarette lighter (disposable/striker and body)	Paper (hand-folded [3 folds in paper for mailing])
	Cigarette paper	Pen (Bank robbery–roped pen owned by bank)
	Control levers (for signal lights etc–automobile)	Plastic bag handles
	Dime	Pry bar with shoulder straps
	Door bell	Remote car starter
	Door pull	Rope
	Drug syringe barrel exterior	Screwdriver handle
	Electrical cord	Seat belt buckle (automobile)
	Expended .22 calibre cartridges and rifle	Shoe laces
	trigger, scope, stock, and barrel	Steering wheels
	Fingerprint (Single)	Tape on club handle (not only exposed surface
	Gauze and tape (used to cover fingertips)	but also initial start under layers of tape)
	Gloves (interior [finger tips & cloth] and exterior)	Toy gun (overall)
	Hammer (head and handle)	Wiener
Mouth and Nose	Apple core–bite marks	Lipstick (top surface and outer surface of lipstick case)
	Balaclava (knitted cap)	Nasal secretions (tissue)
	Bite marks	Peach strudel
	Bottle top	Pop cans/bottles
	Buccal stick only (swab entirely cut off previously)	Ski coat collar
	Cake (bite mark)	Salami (bite mark)
	Cheesecake (bite mark)	Stamps
	Chicken wing	Straws (from drinking glass)
	Chocolate bar (bite mark)	Telephone receiver
	Cigarette butts	Tooth
	Envelopes	Toothbrush
	Glass rim	Toothpick
	Gum	Vomit (bile-like sputum/liquid)
	Ham (bite mark)	Welding goggles (rim of eye/nose area)
General Body	Baseball cap (swab of inside rim)	Head-rest (automobile)
	Bullet hole in gyproc wall and bullet	Paraffin embedded tissue
	Buried remains	Razor (disposable type/blade and plastic cap)
	Burned remains	Tissue paper wiping of underarms of shirt—(sweat)
	Chain (approx 60 cm of end of automobile chain–no	Socks
	blood found, but alleged to have been used to drag body)	Toilet–knife found in "toilet trap"
	Hair	Urine in snow
	Hair comb (for head hair)	Water–"S" trap of shower
Sexual	Automatic machine washed blue jeans (crotch for semen)	Inside edge of fly of undershorts
	Inside undershorts	Pubic hair comb (from sexual assault kit–white
		cotton fibre material)
Eyes	Contact lens fragments (from vacuum cleaner bag)	Eyeglasses (ear and nose pieces)
		Tears (on tissue)

The term "trace DNA" is used throughout this paper. Trace DNA is meant to describe the minute quantities of DNA transferred through skin contact, which can be successfully analyzed and follow the general principles of trace evidence. The small number and nature of these transferred DNA bearing cells make identification of cellular source of origin either impractical or impossible, but through the increased sensitivity of STR PCR DNA typing, DNA profiles are routinely obtained.

Discussion of Selected Cases

Closer examination of a number of these cases illustrates more than just the association of the suspect to a weapon or to a scene. Specific cases also offer some insight into the nature of trace DNA, and some of the considerations entailed in its application.

Case A

In a murder case, the victim was reported missing by relatives. The victim's car was found abandoned in a downtown area, three days after the last sighting of the missing person. Epithelial cells were recovered through swabbing of the steering wheel of a car. The DNA profile obtained from the swabbing of the steering wheel was an obvious mixture of two individuals. The major DNA profile was the same as the DNA profile from the suspect, and the minor DNA profile was the same as the victim (28). In a subsequent limited study of DNA profiles generated from steering wheels, it was determined that the major profile was always that of the last driver (29). This was true even in cases where the last driver was not the regular user of the car. This case and subsequent study demonstrate that trace DNA is left behind from the last individual to contact the substrate, although mixed profiles including previous handlers often result.

One interesting exception to the statement in the previous paragraph regarding order of contact exists. If the last driver of a car is a suspect is wearing gloves, the suspect's profile would not be expected to be seen on the steering wheel, assuming the exterior surface of the gloves is not acting as a vector for DNA carried from a "non-hand" area of the suspect's body (ruling out suspect's saliva, blood, wiped sweat from brow, etc.). The anticipated effect is that loosely adhering cells would be removed or "cleaned" from the substrate through active contact with the non-DNA cell bearing external surface of the gloves. If there are a number of cells loosely adhering to the steering wheel prior to contact with the suspect's gloves, one would expect the same resulting DNA profile after the glove contact as before the glove contact, but now lower in magnitude, if a DNA profile is recovered at all. If those cells (and subsequently that DNA profile) were a mixture of more than one individual, one would expect that they be removed in the same ratio that they are present. In fact, the cells from the two different individuals should be further blended by interaction with the exterior surface of the gloves, such that a more homogenous mixture of cells would result at different areas around the circumference of the steering wheel, should the steering wheel be divided into areas and sub-sampled. In conclusion, outside of a few improbable possibilities, if a suspect is wearing gloves, no forensic association between the suspect and the steering wheel is likely.

Case B

An armed robber entered a bank, and used the pen provided at the bank counter to write a hold-up note. His activity was caught on surveillance camera, but he was sufficiently disguised to escape identification. The pen was examined, swabbed, and processed for trace DNA. The resulting profile demonstrated a major profile matching the suspect, which was easily separated from a number of minor trace profiles (30).

The pen at the bank represents one of the worst possible substrates in terms of potential for an uninterpretable mixed profile from a large number of contributors. Yet, the suspect's contact effectively "replaced" DNA bearing cells previously deposited on the pen, while depositing his own. This case demonstrates the theory that the DNA bearing cells are loosely adhering to the substrate, and that the previous contributor will often be replaced by subsequent contact. Each contact leaves a substrate with a number of DNA bearing cells. A subsequent contact will possibly change the number of these pre-existing cells, by possibly removing cells, as well as adding his/her own, thereby re-establishing a new equilibrium. As in Case A, a trace DNA profile may indicate the last individual to contact the substrate.

Case C

A sexual assault case was investigated which involved a sexual assault with a hot dog (smoked sausage). Each end of the hot dog was swabbed separately. One end revealed the complainant's profile, while the "handled" end demonstrated a mixture of the suspect and complainant (28). This case serves to demonstrate the potential for one exhibit to bear more than one significant profile relative to their location on the substrate. Therefore, exhibits may be considered for sub-division into zones of potential contact and sub-sampled accordingly, in order to maximize their evidentiary value.

Case D

In a murder case, the means of strangulation was the black rubber electrical cord from a vacuum cleaner. The scientific examiner broke down the electrical cord into four zones of potential contact. Each zone was swabbed, and processed individually. Not surprisingly, the central zones yielded DNA profiles matching the victim. The zones to the outside ends of the electrical cord exhibited mixed profiles, which included that of the suspect (31). As in Case C above, this case illustrates the need to treat areas of a single exhibit separately. Mixed profiles can be minimized by attempting to separate individual contributors through logical sub-sampling. Furthermore, areas with large amount of victim's DNA will be less likely to mask offender's DNA in a mixed profile if their respective DNA can be sampled and analyzed separately.

Case E

A female was tricked into assisting a male to an apartment. She was subsequently forcibly confined in the apartment where she was sexually assaulted and beaten. During the struggle, she lost a contact lens. She managed an escape after several hours, but did not inform police until several days had passed. Upon their attendance at the scene, police learned the suspect was watching the apartment for a friend, and had cleaned and vacuumed the scene. The vacuum cleaner bag was searched, and fragments of contact lens located. DNA analysis yielded a profile matching the victim (32). In this case, the vacuum cleaner bag represented a very large potential for additional profiles to that of the victim, yet these additional profiles were found to be only present as trace contributors. This case demonstrates that DNA bearing cells can adhere well to a good substrate, endure environmental insult, and potential contamination with foreign profiles, yet still produce a dominant profile.

Case F

A pair of gloves was inadvertently left behind at a crime scene. The interiors of the gloves were sampled, and DNA profiles were obtained. These profiles were matched back to the wearer, who was a suspect in the offence (33). Interestingly, the known profile was obtained from vomit. This case illustrates that points of contact within worn items can yield profiles of the wearer. Care must be taken, however, in that an individual may "borrow" an item for a one-time use. Also, if biological materials are present on one side of a worn garment, the wearer's profile may be present on the reverse side. Careful separation and sampling of material as opposed to making cut outs may be warranted, depending on the nature of the deposited material. Swabbing is still an option on cloth, particularly non-adsorbent synthetic materials.

These cases serve to illustrate the transient nature of adhering cells, in that they can be rubbed off, and replaced. Despite the illustrations of a large number and variety of successful cases demonstrated, a large number of exhibits examined do not produce a DNA profile. In the author's experience, a trace DNA profile is obtained for approximately 30 to 50% of exhibits tested; using the same routine standardized STR PCR DNA typing protocols as are currently utilized for more traditional biological stains (32). As a result, any laboratory currently using validated STR PCR DNA procedures can implement trace DNA profiling without varying significantly from their current protocols. Use of DNA quantification systems should not be used as a screening tool for trace DNA. Trace DNA profiles are routinely produced from samples where no quantification result is produced. While success in every case is far from guaranteed, these cases serve to illustrate the tremendous forensic potential of PCR STR DNA profiling of skin cells transferred by contact and handling, through the large variety of objects involved in commission of crime.

Discussion of Theory and Application

The potential use of sloughed epithelial cells as a source of questioned DNA for forensic comparison in routine casework has been demonstrated (25–27, Table 1). Although STR PCR DNA typing may produce a complete DNA profile with a very small number of nucleated cells, several hundred or thousand cells may be contributing to the resulting profiles. The cellular origin of these DNA bearing cells can only be speculated in the absence of some confirmatory testing, such as identification of blood or semen. Intuitively, epithelial cells sloughed through active handling onto a porous and jagged substrate (at the microscopic level), should comprise a good portion of the DNA yielding cells. The skin is the largest organ of the human body, accounting for 15% of total body weight (34). The average skin cell spends about one month on the outer epidermis prior to shedding, with the average human being shedding approximately 400,000 skin cells daily. Each square centimetre of skin contains 100 sweat glands, and 10 oil glands (34). Secretions produced within these glands make their way to the skin surface through ducts and pores, thereby exposing them to large numbers of DNA bearing cells enroute to the skin's surface. These cells represent additional potential DNA sources aside from the large numbers of skin cells shed daily. Skin cells are nucleated, and each human cell contains about 5 picograms of nuclear DNA (35). Currently, multiplex PCR DNA type profiling routinely produces full profiles at or below 100 picograms of purified DNA. Therefore, as few as 20 cells will be sufficient to produce a DNA type profile. The skin surface represents a large potential for a source of DNA profiles.

The Locard exchange principle (36) dictates that where two objects come into contact, there is exchange of material. This is the essence of the science of fingerprints, as it is the oil and sweat that is transferred to the substrate surface contacted by the fingers. The fingers act as the vector of transmission. The sweat and oil comprise the transferred material. The image or impression of the fingerprint ridge detail is the information-bearing component of the exchange. This print, or image of the donor's fingerprint, is visualized by a number of means, including use of various fingerprint powders, cyanoacrylate fuming, and metal deposition techniques. Through use of these techniques, reagents preferentially adhere/react to this sweat and oil mixture transferred from the donors skin, to the contacted substrate. The sweat and oil transferred in fingerprints may also be transporting the DNA bearing cells producing the contact DNA profiles found. With the large number of nucleated skin cells available for transfer, and the small number required to produce a profile, it is reasonable to conclude that many items contacting skin during the commission of a crime bear potential for the development of trace DNA type profiles.

Other possible explanations for potential trace DNA sources exist, aside from shed skin cells. The hands may act as vectors of transmission for cells from other body areas (37). These areas include the mouth, nose, and eyes. For example, the cells of both the corneal epithelium (eyeball) and the bulbar epithelium (interior of eyelids and edges of eyeball) are nucleated, and regenerated continuously; being totally replaced every 6 to 24 hours (38). As such, both are potential sources for the DNA found. Rubbing one's eyes may effectively "load" the hands with DNA bearing cells for transfer. Case experience bears out the DNA potential of the eyes as a DNA source as DNA recovered from contact lens fragments has been used to identify the suspect in a brutal sexual assault/forcible confinement case (32). Likewise, rubbing the face, nose, and mouth, chewing fingernails, and other unconscious acts, may be providing a large number of nucleated cells on the hands available for transfer to the next contacted object. The hands and fingers, therefore, may act as vectors of transmission for other cell types, in addition to cells originating from the hands.

Studies were conducted involving the nature of the DNA transfer, including the potential for secondary transfer. Bellefeuille et al. (39) confirmed the potential for DNA transfer from an individual to a surface, but further demonstrated the transfer from the surface back to the individual. A subsequent study by Ladd et al. (1999) demonstrated that secondary transfer of DNA did not occur with sufficient frequency to be a major concern in casework applications (40). A very small number of DNA bearing cells are involved in a detectable primary transfer, estimated to range from 20 to 1000 cells. A detectable secondary transfer is very unlikely, as a number of these cells will adhere to the second individual, or be lost, leaving very few cells available for a secondary transfer. Further, the second "vector" individual is composed of the same DNA as the original DNA subject to secondary transfer. If any secondary transfer was to occur, the individual acting as the vector of transmission is a very large source of DNA bearing cells and would contribute to the production of a mixed profile, within which that vector individual would be the major contributor. Therefore the fraction of the 20 to 1000 trace DNA cells possibly transferred through secondary contact are in a very small ratio relative to this second individual, and would certainly represent a minor component in a mixture, if enough to be detected at all. In conclusion, it is extremely unlikely for the vector individual to inadvertently transfer only the first person's DNA without also leaving his or her own in a larger amount.

A number of factors, aside from the number of DNA bearing cells available for transfer, may contribute to the overall opportunity for success in obtaining a DNA profile on the handled substrate. Handling time, the substrate surface being contacted, the time of contact, and environmental factors all affect the amount and quality of DNA available for analysis. Finally, recognition of exhibit potential, and optimal preservation, collection, and processing of exhibit material, will increase opportunity for success.

The relationship between the amount of DNA transferred to a substrate during handling and (a) the handling time, (b) the individual handler, and (c) the handled substrate, was examined in a study conducted by Kisilevsky and Wickenheiser (41).

The potential to generate full DNA profiles from sloughed epithelial cells, from various substrates at a variety of handling times, was addressed in this study.

It was found that the amount of DNA transferred to a substrate during handling is:

1. Independent of handling time. Transfer of DNA from sloughed epithelial cells, to a substrate during handling, is instantaneous. DNA type profiles were obtained in as little as 10 seconds of contact in favorable conditions (see (2) and (3) below).
2. Dependent on the individual handler. Certain individuals are "good" epithelial cell donors ("sloughers"), while other individuals are "poor" epithelial cell donors ("non-sloughers").
3. Dependent on the handled substrate. Porous substrates adhere sloughed epithelial cells more readily than non-porous substrates.

The potential generation of a full DNA profile is maximized by a "good" epithelial cell donor ("slougher") handling a porous substrate.

It is interesting to note that those surfaces traditionally found to be good substrates for fingerprint transfer and visualization, are smooth and non-porous, such as glass and polished metal, are poor substrates for trace DNA recovery. Conversely, surfaces, which have been found to be a challenge in recovering good fingerprints, such as rough substrates like unpainted wood, are good substrates for trace DNA recovery. A study by Zamir et al. (42) using adhesive tape, coupled with DNA type profiles recovered from adhesive tape at crime scenes by Hummel (28) both demonstrate the potential for trace DNA performed in conjunction with traditional fingerprinting protocols, and the requirement for special handling, determinant on the exhibit type.

Therefore, in the process of maximizing evidentiary value of an exhibit, the examiner must consider the surface examined, and the likelihood of obtaining a useful fingerprint, versus a trace DNA type profile. Surfaces that have a very rough and or broken surface, such as the knurled handle or hammer of a revolver or a rough wooden handled steak knife, may be directly swabbed for trace DNA. A smooth handled weapon or glass may be processed for fingerprints initially. Should prints be found and matched, DNA processing may be precluded. Should unusable smudged prints be found, however, DNA type profiling can be then used without reservation. Items that can be cut out and placed in appropriate test tubes for DNA extraction and purification, such as tape and fabric, may be processed directly or swabbed (See Appendix).

Trace DNA profiles have been developed, even after processing for fingerprints, using cyanoacrylate fuming and metal deposition techniques on a variety of items, including knife handles (24). It is speculated that rather than jeopardise recovery of DNA bearing cells, the application of a very thin layer of acrylic through the fingerprint fuming process may help seal cells in place, to be removed later though swabbing. Conversely, the rough wooden handles on the knives (24) may have resulted in very poor adhesion of fingerprint reagents, making their use "non-DNA-destructive." A number of studies have been conducted regarding the effects of fingerprinting agents on blood typing and DNA (43–51). Generally, limited exposure to fingerprinting chemicals constitutes a manageable risk to the processing of DNA itself. The concerns expressed herein include the potential dislodging and removal of trace DNA bearing cells in the fingerprint enhancement process. In light of these results, and the mechanical nature of traditional mechanical dusting for fingerprints, it is recommended that cyanoacrylate fuming and metal deposition techniques be the methods of choice for non-DNA-destructive fingerprint examination. The mechanical action of applying fingerprint powders prior to cyanoacrylate fuming is likely to dislodge adhering DNA bearing cells, and is therefore discouraged.

Increasing the number of potential sources of DNA that can produce forensically significant profiles increases the need for care in exhibit handling. One exhibit very often harbors DNA from more than one source. In many cases this is ideal, as the sources may be the complainant/deceased, and the perpetrator of the crime. Separating mixed samples can be a tedious mathematical process, which invariably reduces the confidence level in which each individual in question can be declared a potential contributor to the mixture. Ideally, if individual samples can be taken from significant areas with different sources of DNA, mixtures can be reduced, or eliminated. This would allow for far simpler forensic interpretation. Also, greater forensic significance is attributable to findings, as the original context of DNA bearing cells can be considered.

In order that the forensic significance of evidence be maximized through minimizing mixtures, and retaining the original context of DNA deposited on an exhibit, orders of magnitude of DNA bearing cells should be considered. That is, presence of materials bearing very high levels of DNA is correspondingly far greater threats to overwhelming those with much less relative quantities of DNA. As a result, the potential of each exhibit for various DNA bearing cells must be evaluated, and consideration given to retain the original context.

An approach will be suggested herein to assist the forensic investigator in maximizing this evidentiary value. It should be noted that each case must be viewed individually, and decisions made based on specific case circumstances, appropriate laboratory policies and procedures, and individual training and experience. In trace DNA cases, the cellular origin of cells is not identified, and the actual number of cells being used is not established. In fact, trying to determine these facts may consume valuable sample and thereby jeopardize obtaining a DNA profile, which yields far more probative information than the type and number of cells analyzed. Mixtures of cell types may also be present, further indicating the necessity for a case-by-case approach. However, given these limitations, the suggested approach presented can be of value for forensic DNA examiners in making successful choices regarding sample analysis.

Toward this end, potential sources of DNA may be categorized according to their relative quantities of DNA (See Table 2). The amount of DNA borne per volume of sample material or exhibit encountered varies depending on the source. "Solid" samples of DNA, such as tissue blowback from a shotgun, or as found on the blade of a knife, bear very large amounts of DNA per unit volume. Likewise sperm contains very large quantities of DNA per unit volume, as they have been described as "bundles of DNA with tails." Therefore, samples of this type fit into Category I in terms of DNA

TABLE 2—*System for categorization of DNA bearing sources.*

Category	I	II	III	IV
Body Origin	Tissue, semen	Blood	Saliva, nose, mouth, worn items	Trace DNA
Evidence Types	Blowback from explosion, firearm discharge, tissue on knife, weapon, or clothing, used condom.	Clothing, weapons, etc.	Facemask, tissue, glassware, utensils.	Weapon handles, clothing contacting skin, and handled objects.
Suggested order of Removal	Last	Third	Second	First

source potential. Blood is an excellent source of DNA due to its presence in many cases of violent crime, however the DNA bearing white blood cells are in a distinct minority relative to the unit quantity of blood. The roughly 400:1 red blood cell to white blood cell ratio places blood at the second level of DNA sources, or Category II in terms of volume to DNA yield. Next, saliva and items in contact with the mouth and nose are excellent potential sources of DNA. Facemasks, coffee mugs and glasses, cigarette butts, drinking straws and the like, all routinely yield DNA profiles. The amount transferred per exhibit item is usually very small, due to the small volume of body fluid conveying the DNA bearing cells and small contact area. There are a reduced number of cells present, particularly in comparison to blood, for instance. Therefore, saliva, and nose and mouth contact would be placed in Category III. Finally, trace DNA should be evaluated in relation to other DNA sources. Given its transient nature, in that not all substrates contacted will possess sufficient surface adherence to hold sufficient cells to produce a DNA profile, and not all donors will shed sufficient DNA to produce a profile, trace DNA is classified as Category IV.

In order to minimize the potential masking of suspect profiles, particularly in trace DNA, it is necessary to consider the categories of other potential sources of DNA found on the exhibit in question. For, example, blow-back of Category I tissue from the victim shot at close range, would be of grave concern in relation to the potential for Category IV trace DNA on the knurled handle or hammer of the suspect weapon. Careful separation and treatment of these areas are dictated in order to maximize the potential to obtain pure profiles from each individual in their original respective position on the weapon. Likewise, separate handling and sampling of the Category II bloodied blade of a knife relative to the Category IV handle is warranted. A third example is light smudges of suspect blood (small amount of Category II) in relation to the garment wearer's profile (large amount of Category IV). The cuff of a sock belonging to a deceased yielded the wear's profile at every sampling, while the suspect's DNA was captured only when a light smudge of blood was included. In this case, careful separation, swabbing, or scalpel shaving of the sides of the fabric may have reduced or eliminated wearer's profile in the mixture. Development of a DNA profile from the wearer of a garment (Category IV), which has complainant/deceased DNA on the bloodied (large amount of Category II) exterior, such as from the interior of a bloody glove dropped at a murder scene, could establish the identity of the wearer. Separate sampling of interior and exterior is highly recommended in samples of this type.

Dried blood (Category II) represents a special problem in handling with regards to trace DNA. Dried blood often reaches a powdery consistency, and loses adherence from substrates, while held prior to examination. These blood flakes are susceptible to static, particularly when packaged in plastic. As a result of handling, blood flakes originating from a victim may serve as a potential contaminant to suspect trace DNA (Category IV) on a weapon handle. Personal experience of the author has shown that this potential "contaminant," frequently overwhelms the trace DNA. A mixed profile often results, with the complainant/deceased representing the major contributor, and the suspect trace DNA masked as a minor contributor. Minor alleles may be lost in stutter bands of the major contributor, or within shared alleles. Precautions can be taken to minimize this potential. Bloodied ends of weapons should be packaged separately from handles, in order to prevent mixtures through cross contamination. This would involve the use of two smaller bags, tied at a point central on the weapon, separating the two areas of competing DNA. Use of porous, static resistant materials for packaging, such as paper or fabric, may reduce both static and moisture buildup. Maintaining a dry exhibit is critical to limit bacterial and fungal growth, and therefore preserve DNA while in storage.

High yielding DNA Category I sources should be processed last, versus high potential areas for Category IV trace DNA. The rationale behind this approach is that any additional disturbance of the major contributor(s) of DNA will jeopardize opportunities for mixture free collection. Swabbing or other collection methods may serve to dislodge and distribute the major DNA Category I contributors, thereby increasing the risk of masking minor Category IV contributors in mixtures. Therefore, it is recommended that exhibits be evaluated for trace DNA potential, and probative Category IV (trace DNA) exhibit areas be swabbed first (see method in Appendix), followed by Category III, Category II, and finally Category I. Care should also be taken in handling, not to touch higher Category areas, and then lower Category areas, thereby inadvertently transferred material during examination.

The DNA examiner may chose to combine several areas with trace DNA potential on one single swab. An example would be the trigger, hammer, and burled grip of a gun. There is a reasonable assumption that the same individual may have handled all three of these areas, and there is a minimal risk of creating a mixed profile by combining separate areas. Based on the roughness and size of the surface area to be swabbed, each of the three areas may be assessed as to their potential relative to each other. Areas of lower potential should be swabbed first, and areas of highest potential swabbed last. In this manner, the risk of loosing cells from the swab back to the substrate is minimized.

Great care must be taken in speculating the somatic origin of DNA bearing cells found on exhibits where trace DNA profiles have been developed, yet the originating cell source not determined. Dependant on case circumstances, minute undetected quantities of blood, saliva, semen, or other bodily sources, or even mix-

tures of these, can be the origin of the DNA profile(s) seen. A study conducted by Jobin and DeGouffe (52) recovered DNA profiles from seminal fluid stains on cotton and polyester material that had been machine-washed. In all cases, the traditional acid phosphatase screening test for seminal fluid was negative, yet sufficient sperm were retained to produce DNA type profiles. Furthermore, samples taken surrounding the known areas of semen deposition always remained negative, demonstrating no secondary transfer, even in machine washing. Despite the need for conservatism in stating positive conclusions regarding somatic origin of DNA yielding cells, one statement can be still made with full confidence. That is that the exhibit in question has been in contact with cellular material originating from an individual with the DNA type profile developed.

Even with the increased potential for associating a suspect with a crime scene, the absence of evidence does not constitute evidence of absence. That is, not finding a suspect's DNA profile at a crime scene does not prove that they were not present. As discussed, there is much potential for finding a suspect's DNA present at a crime scene. There are, however, reasons why a suspect may have been present at the crime scene, yet a forensic association not established. Insufficient DNA may have been transferred to exhibits to produce a profile. Areas of contact may have been overlooked, or contact simply not taken place. Therefore, finding or not finding DNA is a one-way proposition. Finding DNA indicates contact, while lack of a DNA profile is inconclusive.

As the sensitivity of multiplex STR PCR DNA profiling sensitivity increases, with less and less DNA required for the development of a DNA profile, the "Forensic Context" of DNA recovered at scenes of crime must be closely scrutinized. A DNA profile found at a crime scene, which is indistinguishable from a suspect, often shows only association. Time and context of the contact often must be demonstrated through further investigation and other forensic evidence. Although case experience has found that the handled object bears the profile of the most recent handler, many more mixed profiles will be recovered if commonly handled objects are examined. Doorknobs and handles, light switches, ignition switches, and doorbells have all yielded DNA profiles (25–27, Table 1), yet represent potential "red herrings" in terms of an investigation if taken out of proper context. DNA profiles may have originated from an individual with an innocent reason for being present at the crime scene, rather than relate to the perpetrator of the crime in question. Likewise, panties have been demonstrated to harbor sufficient sperm to produce a full DNA profile, even after washing (52,53). If taken out of context, one could easily eliminate the true perpetrator by developing a DNA profile, which did not in fact have anything to do with the offense in question. The profile may have originated from a previous consensual sexual partner not related to the offense. Additionally, two cases were reported where full male suspect DNA profiles were recovered from sexual assaults involving vasectomized males (54). Various scenarios involving multiple suspects or sexual partners could restrict the value of a positive DNA finding in conclusively eliminating or incriminating a suspect. Therefore, only after a thorough examination of the known facts surrounding a case, and a multidisciplinary forensic investigation, should conclusions be drawn.

When a questioned (suspect) DNA profile is indistinguishable from a known (reference) source, an opinion as to the likely source of origin may be proffered. Assessing the evidentiary value of DNA profile "matches" has long been a topic for debate. It is generally conceded that once a DNA profile is generated, the chances of randomly selecting a matching profile from an unrelated individual are extremely remote. This does not consider the "forensic context" of the finding, however. Establishment of an opinion regarding the significance of forensic findings should include consideration of the following features:

1. Access—The suspect population is confined to those with access to the area.
2. Transfer—The suspect must shed the DNA bearing cells at the scene, in a significant location. These cells must then be recovered in sufficient amount and condition in order to generate interpretable findings.
3. Discrimination power—The DNA profile generated by the suspect known sample must be included in the DNA profile generated by these questioned cells. This point is represented by the estimated frequency of occurrence, usually obtained from a number of suspect population databases.

In most cases utilizing current multiplex STR PCR DNA profiling, the combined estimated frequency of occurrence in the suspect population represents an astronomically rare number. When the forensic context is considered along with the estimated frequency of occurrence, a very strong opinion may be stated regarding the origin of the DNA profile generated from a forensically significant exhibit. This strength of this opinion may be reduced accordingly with a decreased number and discriminating power of loci available, should a full profile not be developed.

As evidence given at trial by a qualified expert is opinion evidence, at some point the opinion offered will be common identity between the questioned and known sources. This point will be up to the qualified examiner, considering the DNA evidence at hand, combined with the forensic context, training, and experience. While opinions are subjective, and circumstances vary considerably, an indistinguishable profile within the appropriate forensic context found in the 13 core loci used in CODIS (Combined DNA Index System - FBI) would be considered reasonable proof of identity by the author.

Despite the demonstrably huge potential of trace DNA, there lies additional yet largely unrealized benefit in DNA type profiling in general. The known offender DNA Databank in England now nears one million known profiles, and boasts not only 900 plus hits per week, but also a success rate of over 40% per query (55). The National DNA Index System (NDIS) has been operational in USA since October 1998, and contains approximately 760,000 profiles as of September 1, 2001. There have been 1000 investigations aided in the first nine months of 2001, and that number is increasing exponentially as the number of offender samples in the database rises as states come on line (56). Success in known offender databases rely on recidivism; that is the same offender continues to commit multiple crimes, the severity of which often increases in nature. Social scientists claim a very impressive rate in reversing this trend, with an estimated 70 to 80% success rate, provided that the offending individual can be accessed and corrected early in their "career" (57). The real potential in the application of forensic DNA may be in giving early accountability, and serving to highlight youths at an earlier, more correctable stage in their development. One of England's program goals is to realize a one-week DNA response time for property offences, including stolen cars, often via the swabbing of steering wheels. A subsequent goal is to reduce auto thefts by 30% in three years. Application of forensic DNA early in a crime investigation, and also early in the correctable life of an offender, serves to maximize this benefit to society.

Conclusions

1. Trace DNA bearing cells are loosely adhering to contacted substrates. The previous contributor will often be replaced by subsequent contact by a second individual. A trace DNA profile is indicative of the last individual to contact the substrate.
2. One exhibit may bear more than one significant profile relative to their respective locations on the substrate. Therefore, exhibits must be broken down into zones of potential contact and subsampled accordingly, in order to retain the original forensic context of DNA profiles, in order to maximize their evidentiary value.
3. While secondary transfer of trace DNA is possible, the transferred DNA will be overwhelmed by the vector individual's DNA, or be a minor component in a mixed profile. It is extremely unlikely for the vector individual to inadvertently transfer only the first person's DNA, without also leaving his or her own DNA in a larger amount.
4. Care must be taken while sampling areas with potential for more than one source of DNA bearing cells. A technique is proposed herein to maximize evidentiary value through minimizing mixtures, while maintaining the original forensic context of DNA bearing cells.
5. Maximizing evidentiary value of trace DNA evidence requires a co-operative approach with Fingerprint/Identification Specialists, utilizing non-destructive cyanoacrylate fuming techniques.
6. Various scenarios could restrict the value of a positive DNA finding in conclusively eliminating or incriminating a suspect. Therefore, only after a thorough examination of the known facts surrounding a case, and a full forensic investigation, should conclusions be drawn. Once a profile has been declared indistinguishable within the appropriate forensic context, a positive conclusion regarding origin can be drawn, in the opinion of the author.

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Appendix—Technique for Swabbing for Trace DNA

1. Determine the order of analysis of probative exhibits in order to maximize evidentiary value while minimizing evidence destruction/consumption. Record any impressions, fingerprints, or other perishable information that will be destroyed by swabbing the substrate surface. Photographic images including a scale are preferred.
2. Exercise contamination prevention by wearing gloves, a facemask if sniffling or sneezing is a concern, etc. As DNA bearing cells may be loosely adhering to suspect surfaces, take care to minimize handling or contact with areas to be swabbed.
3. Thoroughly wet sterile cotton swab with distilled water. Shake off excess water. Cotton swabs are highly recommended over synthetic materials, as they are hydrophilic, and much more irregular in surface texture. Cells have been found to adhere far better to cotton than synthetic fibers (52).
4. Swab the substrate aggressively, rotating swab into direction of swabbing action, utilizing tip and full sides of swab. The process of swabbing breaks down into the following three phases:
5. Wetting: The substrate surface is wet by the solvent, distilled, or de-ionized water. The flow of solvent is away from the swab, onto the substrate, until equilibrium is reached between the swab and the substrate.
6. Evaporation: As the substrate is swabbed, the reservoir of solvent on the swab is exhausted. The flow of solvent from the swab onto the substrate ceases.
7. Capillary action: The cotton fiber is hydrophilic, absorbing and holding up to 14% of its weight in water. Also, the fibrous nature of the swab itself causes re-absorption of solvent back into the swab as the swab and surface dries, via capillary action.
   Therefore, in order to maximize the removal of suspect material from the substrate, surfaces should be swabbed until the wet glistening has been removed, and the surface appears nearly dry. Mechanical action is thought to be responsible for the removal of cells bound in acrylonitrile resulting from the fuming process, and likewise, trace metals used in the metal deposition process. The swab should visibly remove these reagents from the suspect surface, and be retained on the swab.
8. Document details regarding exhibit description, and area(s) swabbed.
9. Air-dry swab in cabinet to prevent contamination.
10. Once dried, transfer swab into pre-labeled DNA tube for processing.

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