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Science and Justice, 2020; 60(3):206-215

Journal Pre-proofs

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PII: S1355-0306(19)30292-8
DOI: https://doi.org/10.1016/j.scijus.2020.01.002
Reference: SCIJUS 863

To appear in: *Science & Justice*

Received Date: 8 November 2019
Revised Date: 20 January 2020
Accepted Date: 26 January 2020

Please cite this article as: D.O.M. Bonsu, D. Higgins, J.J. Austin, Forensic touch DNA recovery from metal surfaces – a review, *Science & Justice* (2020), doi: https://doi.org/10.1016/j.scijus.2020.01.002

Forensic touch DNA recovery from metal surfaces – a review

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January 2020

Conflict of Interest

None

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Abstract

Trace evidence such as touch (also known as contact) DNA has probative value as a vital forensic investigative tool that can lead to the identification and apprehension of a criminal. While the volume of touch DNA evidence items submitted to forensic laboratories has significantly increased, recovery and amplification of DNA from these items, especially from metal surfaces, remains challenging. Currently little is understood with regards to the underlying mechanisms of metal-DNA interactions in the context of forensic science and how this may impact on DNA recovery. An increased understanding of these mechanisms would allow optimisation of methods to improve outcomes when sampling these materials. This paper reviews the basis of DNA binding to metal substrates, the merits and limitations of current methods and future perspectives of improving recovery and amplification of touch DNA from metal surfaces of forensic interest.

Keywords: Forensic Science; metals; touch DNA and/or contact DNA; direct PCR; swabbing; tape lifting; Bardole M-vac.

1. Introduction

DNA evidence has become an indispensable tool in forensic investigations globally. Due to advances in DNA extraction and amplification technologies, most biological samples can now be tested to yield critical genetic evidence [1,2]. It is now possible to produce a forensic DNA profile from trace sources, such as touch (also known as contact DNA). Forensic laboratories currently receive numerous requests for touch DNA analysis. These relate to property and violent crimes with no blood or semen, in anticipation that touch DNA testing may provide investigative leads [3]. Further, cold cases where body fluids are absent, or samples had extensively degraded are now being resubmitted for touch DNA analysis [4]. Touch DNA testing is, however, impacted by the difficulty in obtaining not only enough quality DNA to generate a complete DNA profile but also sufficient material to allow re-testing.

Touch DNA evidence results from the transfer of biological material to a substrate upon human handling or contact. Touched surfaces may retain genetic material in many forms including epithelial cells, fragmented cells/nuclei, cell-free DNA [5–7]; and anucleated corneocytes [6,8]. These cells are not visible to the naked eye [9], hence, are typically recovered speculatively. At a crime scene, DNA may be present in very low amounts, so there are practical difficulties in recovering enough nuclear or mitochondrial DNA for typing. DNA is routinely recovered from plastic, glass and fabric surfaces to obtain relevant profiles [10–12]. However, it has proven to be more difficult to consistently recover touch DNA from metal surfaces [13].

Metals are ubiquitous and generally encountered in forensic investigations as part of the built environment (such as window frames and doorknobs), wearable material (such as jewellery, belt hooks, shoe buckles and eyeglass frames), weapons (such as firearms, ammunition, razors, knives and screwdrivers) used in commission of crime or as coatings of other materials. The continual increase in knife (e.g. the UK [14]) and gun-related (e.g. Australia, New Zealand and the USA [15–17]) crimes has, undoubtedly, had a ripple effect on the forensic interest of DNA recovery from such surfaces. Knives, firearms and spent cartridge casings are frequently encountered evidence types in the instance of hate crimes, terror attacks, homicides and wildlife poaching [13,18,19].

Regarding crimes which involve guns (Fig. 1), it may be possible to find touch DNA on the butt, trigger or slide when handled or operated without wearing gloves. Criminals, when attempting to remove evidence, may attempt to clean weapons after use but are probably less likely to wipe the ammunition, which may have been loaded with bare hands. Similarly, a burglar's fingerprints - a source of touch DNA - may, for instance, be left on a brass door handle or aluminium window frame.

Figure 1: **A disassembled pistol with the inside of the hand grip shown**. The red arrows (SP3) indicate the inside surface of the hand grip (a protected area), which was swabbed. The firearm was discarded in a stormwater drain and recovered nine days later following a period of torrential rain. A good DNA profile was obtained after swabbing under the grip (plastic), while other parts of the firearm swabbed yielded no profile. (Picture Courtesy: Dr Jennifer Raymond, Research Coordinator, Forensic and Technical Services Command, NSW Police Force, Australia. Image used with permission).

The recovery and subsequent amplification of DNA is one of the key challenges encountered in the analysis of forensic DNA samples from metal surfaces as a result of nucleic acid – metal interactions. Metals have an array of ionisation and electron affinities that enable their reaction with negatively charged molecules such as DNA [20]. Anastassopoulou [21] , suggested that metal cations interact directly or indirectly with the negatively charged phosphate backbone of

DNA as well as the nitrogen or positive atoms of the nucleobase, allowing the formation of ionic bonds that may impede the release of touch DNA from metal surfaces. This interaction may explain, to some extent, the poor DNA recovery from metal substrates reported by Wood et al. [13] and the presently inconsistent success rate between 0% and 26% noted [22]. This review explores the basis of DNA persistence on metal surfaces and attendant impact on the success of recovery and amplification. It scrutinises the scope and efficiency of current sampling, extraction and direct amplification techniques, and provides relevant recommendations for improving forensic trace DNA recovery from problematic metal surfaces.

2. DNA and metals

The array of ionisation energies and electron affinities of metals impact their degree of interaction with negatively charged molecules such as DNA. Metal cations may interact directly or indirectly with two distinct positions on a DNA molecule: the negatively charged residues site (phosphate backbone) [23] and the characteristic high electron density sites (Nitrogen (N) and Oxygen (O) of atoms of nucleobases) [21]. Pages et al [20] posits that a partially or fully hydrated metal ion exhibits the tightest binding to the hydrated nucleic acid.

The extent of interaction and reactivity of metal ions with DNA is, in part, determined by their position on the Periodic Table. The polymorphic nature and attendant variable structural complexity of DNA offers at least three possible intermolecular interactions intercalation; irreversible covalent binding and groove association [19, 22] (Fig. 2). Generally, alkali metals do not strongly bind to DNA, and their monovalent ions preferentially interact with AT-rich regions of minor grooves [25]. On the other hand, divalent alkali earth metals have a rather high reactivity given their ability to coordinate with mono or bi-dentate ligands and to form basic oxides whose reaction with water yields relatively insoluble hydroxides.

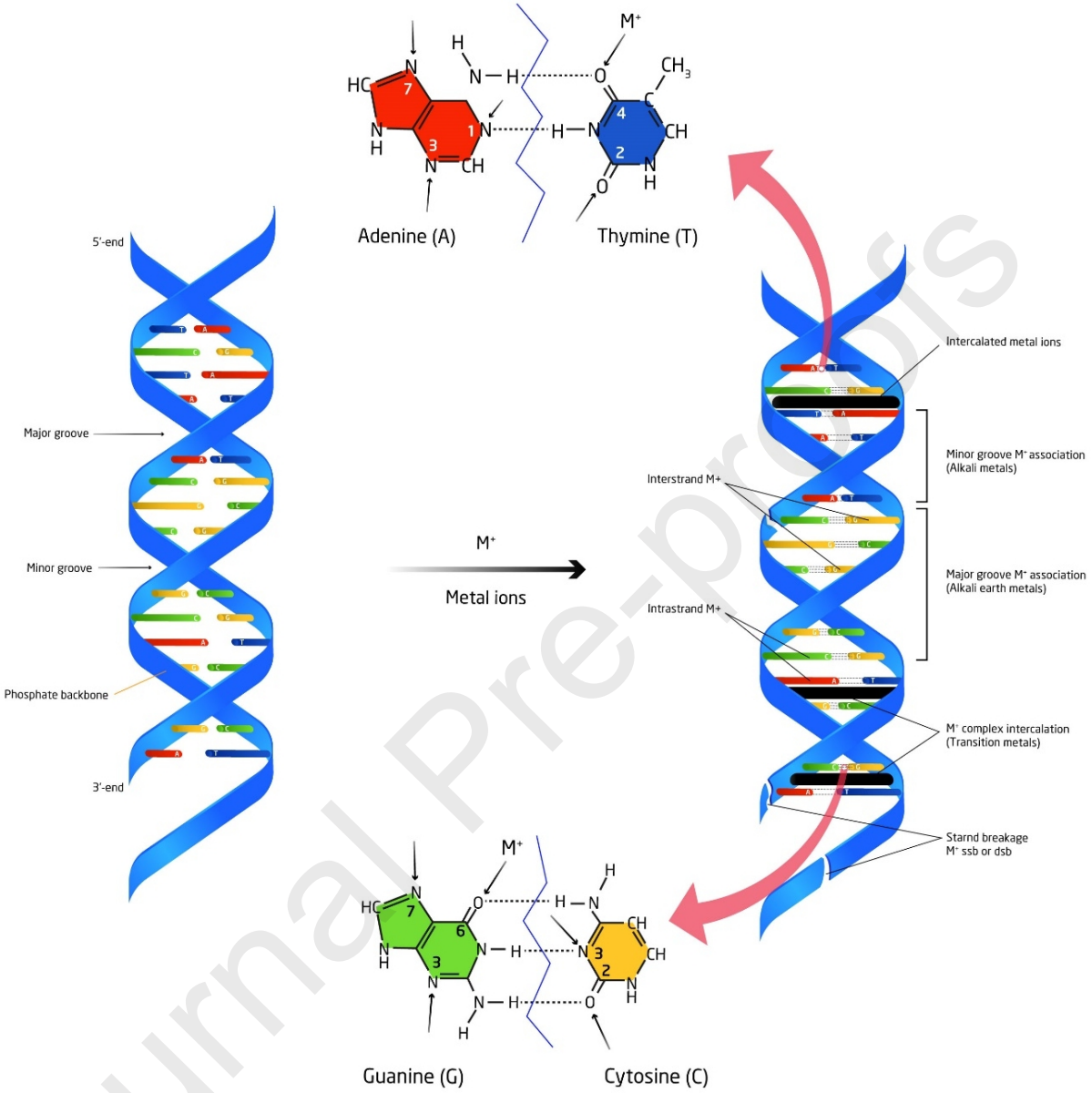


Figure 2: DNA interaction with metals. Ions of metals (M^+) may bind to one or two sites of a single strand (intrastrand) or opposite strands (interstrand), or by complex intercalation between the nucleobases. M^+ binding can cause single strand break (ssb) or double strand breaks (dsb) [21]. Complex intercalation from transition metals can alter the double helix [26], causing damage via oxidative stress when bound to GC rich sites. The M^+ binding sites (arrowed) in Adenine: N1, N3 and N7; Guanine: N3, N7 and O6; Cytosine: N3 and O2 and; Thymine: O2 and O4, disrupts DNA integrity [27].

Ions of magnesium (Mg^{2+}) are, for instance, known to be key intracellular metal ions existing in all nucleic acid (both DNA and RNA) processes of activation, functioning as a link between certain enzymes and nucleotides, nucleosides and their derivatives [28]. Using combined data from X-ray, Fourier-transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectra, the interaction of magnesium ions (Mg^{2+}) with oligonucleotides was reported to primarily occur at the electronegative phosphate group (PO_2^-) [21]; however, the C = O, NH_2, N1, N3 and N7 positions of nucleobase moieties have also been documented as additional binding sites [29–31] (Fig. 2). Hydrated Mg^{2+} is also generally encountered in the major groove located between GC base pairs of specific oligomers [31,32].

DNA is a recognised efficient metal ion chelator as demonstrated by the need for magnesium ions in PCR reactions [33,34]. The chemistry of Mg ions, pertaining to their role in nucleic acid amplification, makes magnesium an important alkali earth metal for studying metal-DNA interaction. As noted by Kornbeg [35], Mg^{2+} is a vital cofactor for all DNA polymerases, including reverse transcriptase. During the polymerisation step of the polymerase chain reaction (PCR) by Taq Polymerase, the 3'-OH of the growing chain contains a lone electron pair which facilitates phosphodiester bond formation. The ensuing nucleophilic attack on the phosphate group of the incoming deoxynucleoside triphosphate (dNTP) releases pyrophosphate (β and γ – phosphates) molecule while bonding the remaining α – phosphate to the O atom on the 3' carbon of the template strand. However, the four negative charges carried on the dNTP overwhelm and retard the nucleophilic attack. Mg^{2+} ions subsequently chelate the extra anions enabling the latter, bond formation and polymerisation [36]. Thus, Mg^{2+} forms Mg-dNTP-complexes with the single nucleotides which then serve as the substrate for polymerase activity in a PCR. The foregoing is the basis for the requirement for an increase in Mg^{2+}concentration when higher than usual quantities of DNA are present in the PCR reaction mixture [34]. A lack of Mg^{2+} leads to no amplification; thus, optimisation of magnesium concentration is routine in most PCR method development. The metal chelating ability may, therefore, contribute to the poor yield of PCR product of samples obtained from metal surfaces, since metal ions and metal-derived contaminants may damage DNA or act as DNA polymerase inhibitors [37,38].

Transition metals present the most complicated interaction due to their ability to form more than one cation with varied ionic charges and subsequent multi-site binding activity with DNA [20]. Through chemical reactions with the N3 atom of pyrimidine (Cytosine or Thymine) or the N7 of purine (Adenine or Guanine), transition metals can alter the double helix [26] and their binding to GC rich sites has been reported to cause *in vivo* oxidative damage to DNA via H_2O_2 generated radicals [39]. The coordinated complexes forming feature of these metals facilitates direct and indirect binding to nucleobases and phosphate groups, respectively [21]. Using Zinc-DNA crystal structural complexes and spectroscopic data, it was postulated that Zn^{2+} tends to bind to "four oxygens of four different phosphates" as well as to the N7 position of guanine base [21]. Copper (II) (Cu^{2+}), Nickel (II) (Ni^{2+}) and Zn^{2+}, albeit different in DNA-binding ability, are known to form complexes with the same ligands due to their qualitatively similar properties and structure [40]. As discovered by Govindaraju *et al.* [41], Cu^{2+} ion binding efficiency is positively correlated with the extent of unwinding of the DNA double helix caused by denaturation, and the metal's redox physiognomies facilitate the generation of reactive oxygen species (ROS) that causes oxidative damage. The latter makes copper a potent antimicrobial surface [42,43] and is probably the cause of the difficulty in collecting sufficient DNA from such surfaces.

Most metals of forensic interest, on account of the difficulties encountered during recovery of DNA and fingerprints in casework, either belong to the transition group or are alloys with at least one transition group component. This is due to the fact that they make up a group of the so-called 'common workhorse' (excepting lead, tin and aluminium) as well as all the 'precious metals' [44]. For example, the alloys: brass (copper and zinc); steel (iron and carbon); and stainless steel (steel plus chromium) are routinely used in the construction of the built-environment and most importantly, the manufacturing of firearms and ammunition. The limited ability to obtain and amplify DNA from brass, an alloy of copper (Cu) and zinc (Zn), for example, has been reported [45–48] and attributed to the physicochemical properties of the copper component of this alloy. Subsequently, copper-induced damage of DNA on fired and unfired cartridge casings have been reported [45,46]. However, other works have reported increased recovery when DNA was directly treated with Cu^{2+} [49], though Cu is expected to inhibit amplification and generation of interpretable short tandem repeat (STR) profiles.

Currently, no available literature has investigated the potential contribution of Zn^{2+} to the limited DNA recovery or inhibition during amplification, although its involvement is possible. More research is thus required to explore the synergies or complementarities of Cu^{2+} and Zn^{2+} metal ion – DNA interactions and the associated effect on recovery and profiling; and to facilitate the development of relevant techniques for efficient nucleic acid amplification.

Numerous studies illustrating the basis of metal-DNA interaction have been reported in the literature [21,40,50]. These studies mostly employ genomic, biophysical and spectroscopic techniques with highly pure, 12 base pair (bp) synthetic oligonucleotides deemed "sufficiently close to real DNA" [28,51], and as realistic models for determination of metal binding sites of DNA. While these works from multi-disciplinary viewpoints make for plausible extrapolations, they are not directly applicable to forensic science and do not precisely represent real-life scenarios for the following reasons. Firstly, the nucleic acids found deposited on metal substrates at crime scenes are mostly complex and typically within a cellular construct (most DNA extraction protocols are optimised to target nucleated cells and rarely utilise cell-free DNA [52]) . The interaction of the other cellular components, such as proteins with metal ions and their influence on the extent of ion accessibility to DNA cannot be fairly juxtaposed with putting the "naked" molecule directly in contact with metal ions, as is the case in experimental setups. The "naked" DNA increases the magnitude and success of metal ion-nucleic acid interaction (due to increased surface-area-to-volume ratio) while discounting the effect of other cellular materials, as is the case in routine forensic scenarios. Secondly, techniques requiring crystallisation (used in studies, e.g. [30,48]) utilise reagents such as 2-Methyl-2,4-pentanediol (MPD), as a dehydrating agent to expel DNA out of solution. The associated dehydration has been documented to enhance DNA interaction with cations, enabling non-preferential binding to any accessible site [25,28]. Thirdly, it is impractical to evaluate the impact of solid metal surface physicochemical characteristics (e.g. texture, the extent of rust) and environmental conditions on recovery and amplification of DNA from research solely focussed on ionic bonding in a strictly controlled *in vitro* setup. Finally, the interaction of metal alloys, which consist of multiple metal ions, with DNA is likely complex but is as yet not elucidated. Whether or not there is inter-ionic competition for DNA binding sites and the scope of impact on extraction and amplification process is of useful forensic research interest.

3. Sampling methods for DNA recovery from metal surfaces of forensic interest

An important aspect of forensic DNA analysis is the collection of trace evidence from the substrate surface. The convention is to use various swab types pre-wet with some buffer or sterile water. The swab is applied to the surface and rubbed using consistent pressure while ensuring maximum swab-surface contact through a measured rotation. It is generally recommended to limit rotation to no more than once in order to avoid compromising the sample through the redeposition of specimen [54,55].

Research targeted at improving trace DNA recovery from problematic metal surfaces centres along: swab type (tip) and/or extraction buffer modification(s); substrate soaking to facilitate solubilisation of DNA into solution for subsequent purification; and tip optimisation for direct sample introduction into amplification systems without conventional extraction [56–59]. Sample collection from metal surfaces can be categorised into five methods, namely: standard swabbing; tape lifting; soaking, the Bardole MVAC and direct PCR (Fig. 3).

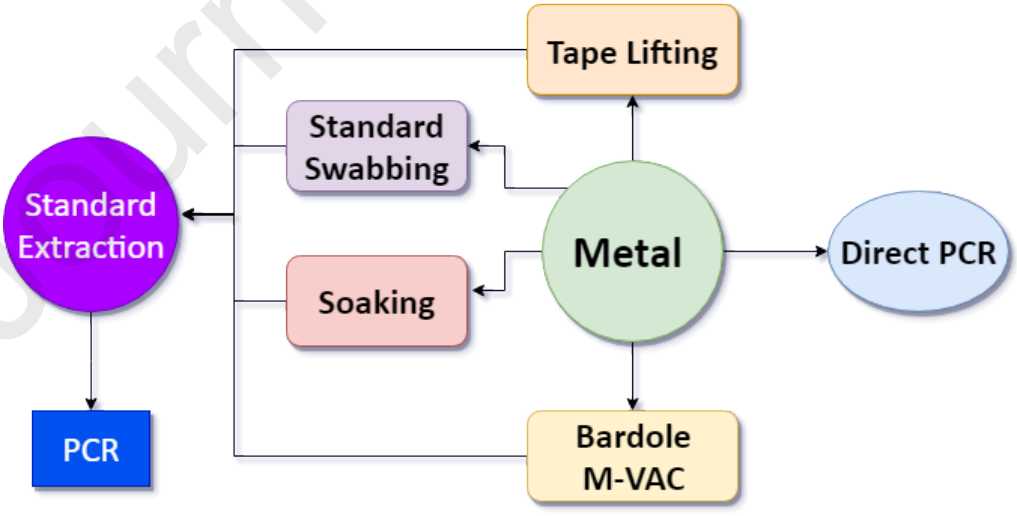


Figure 3: Sampling methods for recovery of DNA from metals (e.g. spent cartridge casings). Current method development is focusing on how best to recover trace DNA from metals with techniques including swabbing, soaking, Bardole M-VAC, lifting with tape or direct PCR —and within those methods, determining which specific techniques are most successful. Excepting direct PCR, the standard extraction process is undertaken after sampling, before conventional DNA amplification via polymerase chain reaction (PCR).

3.1 Standard Swabbing method

Swabs are used in various forensic science settings, and an extensive range is available for DNA sample collection. What constitutes the "standard swab" is a matter of choice based in part on the cost, experience, efficiency, specific in-house (validation) techniques, and compatibility with particular instrumentation. Nonetheless, it appears that the fundamental determinant of the most effective swab device is the substrate on which it is to be used [60,61].

Standard cotton swabs are traditionally preferred for collection of biological fluids (e.g. semen, blood, saliva). Various law enforcement agencies have historically employed cotton swabs as reliable collection devices. This is based on cost-effectiveness, ease of storage, and amenability for high-throughput processing. Furthermore, cotton swabs are simple to use, requiring minimal training for efficient sample collection [54,62]. When trace or touch DNA evidence is envisaged, the double swabbing technique [63] is employed. This method entails an initial wet swab of the sample area, followed by a dry swab aimed at maximising recovery [60,63]. The problems associated with the use and removal of biomaterial from the cotton matrix of swab devices have inspired research into the modification of same or alternative materials to improve evidence collection. Notably, electron micrograph data showed a tendency for trace DNA to get physically trapped and entwined within cotton fibres of swab devices, resulting in significantly reduced efficiency of DNA recovery [64,65].

Lazzarino *et al.* [66] similarly noted that spermatocytes stuck to cotton swabs as a result of sperm membrane saccharic composition, adversely affecting DNA recovery from semen specimens. Furthermore, the occasional inability to generate expected DNA profiles even from DNA rich sources, such as blood, collected with cotton swabs have been reported [56]. This limitation influenced the development of a more efficient alternative, self-saturating foam swabs called mini-popules [67,68], through the collaborative efforts of an Australian forensic

laboratory (Forensic Science South Australia) and Puritan Medical Products Co. [56]. Research for improving swabbing has primarily focused on simplification of specimen collection; maintainence of DNA integrity during storage; reproducibility of cell collection, buffer requirements and compatibility with modern robotic extraction systems, when applicable. For example, it has been demonstrated that, in contrast to cotton swabs, mini-popules have no drying requirement to forestall microbial degradation of sampled DNA; are compatible with robotics and increase trace DNA recovery [67,68].

Isohelix™ swabs are supplied sterilised with ethylene oxide (EtO), hence, they are guaranteed DNA-free, in contrast to the popules. A number of modified sample collection devices such as Dacron, Rayon, FLOQSwabs™, Bode SecurSwab™, and nylon and polyester tipped swabs [69–71], have been developed for trace DNA. These swabs are generally designed to have no internal absorbent core to avoid dispersion and entrapment of the specimen [72], ensuring rapid and complete elution of samples during extraction. There is currently no consistency in swabbing devices used in different forensic laboratories. While a particular swab performs best for non-porous surfaces, it may be ineffective for porous ones. Moreover, the advent of robot-ready tubes may dictate which swabs can be used. Thus, what a laboratory may consider as the most effective swab device is determined primarily by its practicality, as well as the substrate containing the evidence sample. However, none is as yet explicitly acclaimed for touch DNA collection from metal surfaces.

3.1.1 Buffer Solutions

Buffer solutions are integral to conventional swabbing methods and may consist exclusively of deionised water, or deionised water with other constituents, whose functions are related to their chemical compositions. These reagents often include detergents (e.g. Triton-X, sodium dodecyl sulfate (SDS)), a chelating agent (e.g. ethylenediaminetetraacetic acid (EDTA)) or phosphate buffered saline (PBS). EDTA binds metal ions which deplete metals available to metal-dependent enzymes. The resultant ion depletion inactivates enzymes such as deoxyribonuclease (DNase) [73] that could catalyse the hydrolytic cleavage of the phosphodiester bonds, causing DNA damage. SDS, a robust anionic detergent denatures

318 secondary and non-disulfide linked tertiary structures to enhance the release of bound DNA
319 [74].

320 It has become standard practice to moisten swabs when sampling trace biological stains. This
321 facilitates stain rehydration and material transfer to the collection device maximimising the
322 quantity of biological material collected. Deionised water (dH_20) is frequently used for this
323 purpose [75]; however, the hypotonic nature results in cell lysis, releasing DNA that can
324 become entrapped and tangled within swab fibres leading to a decrease in DNA recovery
325 [64,76]. Isotonic PBS offers better rehydration by maintaining cell integrity via its neutral
326 osmotic pressure [77], minimising nucleic acid entrapment during the sampling process and
327 enhancing the quantity of recoverable DNA [76,77]. Buffer solutions can chemically aid
328 solubilisation of nucleic acids from surfaces facilitating adsorption onto the swab and may bind
329 to metal cations that have been released from the surface, minimising the potential for
330 degradation of DNA [78].

331 The type of buffer solution utilised has been reported to be vital to the ability to dislodge and
332 recover trace DNA bound to surfaces [59,62]. In a study comparing effects of multiple buffer
333 solutions on touch DNA samples, Thomasma and Foran [59] found that pre-wetting swabs with
334 buffers containing detergents (Triton-X or SDS) performed better at recovering touch DNA from
335 glass slides than using distilled water only. Similarly, a protocol using phosphate buffered saline
336 (PBS) was successful in the recovery of trace DNA from ridged plastic lids [61]. In a double swab
337 technique using Type I (ultrapure) water as the buffer, Horsman-Hall *et al.* [79] recovered DNA
338 from touched cartridge cases sufficient for STR typing. Phetpeng *et al.* [80] conducted
339 comprehensive research of different swab brands and moistening agents (PBS, sterile H_2O, SDS,
340 ethanol, isopropanol and lysis buffer) for collection of touch DNA from improvised explosive
341 device (IEDs) parts. Their results demonstrated that, while swab types and buffers affect the
342 DNA collection process, there was no individual “best swab brand or moistening agent” and
343 recommended rigorous method validation in each forensic laboratory, to maximise the
344 probative value of trace sample DNA.

345

3.2 Tape Lifting method

The tape lifting technique for the collection of trace biological evidence for subsequent nucleic acid analysis has become a well-established procedure in forensic casework [81]. The technique, intended initially for firearm discharge residues (FDR) recovery [82], is employed in evidence collection from fabrics (e.g. bedding, garments), skin, solid surfaces in vehicle and other crimes scenes and evidence where touch evidence is required [83].

Taping for trace biological evidence with forensic adhesive tapes consists of repeatedly pressing the sticky side (after UV irradiation to remove extraneous DNA) against the material or surface and lifting for subsequent DNA extraction [82,83]. Tapes with stronger adhesion have been reported to give a higher yield of trace DNA than swabbing [55,84,85]. However, the stickiness complicates DNA extraction process [82,83,86], and sampling can be labour intensive [82]. The method has also been adapted for successful trace DNA recovery from ridged metal surfaces [61]. Lawson *et al.* [87] evaluated the effectiveness of tape lifting, submersion and standard swabbing methods on touch DNA from cartridges fired in a revolver including their respective casing. The authors found low quantification values and usable short tandem repeat (STR) profiles were slightly below the laboratory's stochastic threshold and interpretation guidelines, though tape lifting resulted in better DNA recovery than the swabs.

3.3 Soaking method

Soaking or submersion method (also known as the Netherlands soaking method due to its origin) for touch DNA collection and extraction has been explored especially for firearms. The rationale of this technique is that, by submerging the metal harbouring the biomaterial in a lysis buffer, most cells are freed or lysed into solution, afterwards, a dry swab of the metal surface is made to secure residual cell material. The lysis solution and swab are combined for subsequent extraction to increase DNA yield [57].

The proof of concept for this method was advanced by Dieltjes *et al.* [57] in their quest to generate profiles from trace skin cells which are transferred to cartridges, bullets and casings

(CBCs) due to the strong force required for magazine loading in non-military situations. CBCs were soaked in Buffer ATL (lysis buffer of QIAamp® DNA Mini kit), dry swabbed, DNA extracted and amplified with PowerPlex® 16. The authors obtained reproducible profiles in 26.5% of 616 cases and 6.9% of 4,085 individual CBCs examined over six years, showing the potential of the submersion technique for forensic casework. However, it was observed that CBCs underwent oxidation in the ATL buffer, releasing copper ions which turned the lysis buffer blue. Furthermore, CBCs specifically began turning blue when incubated in the lysis solution for a longer time. Montpetit and O'Donnell [88] from the San Diego Police Department Crime Laboratory modified the Netherlands soaking method using an in-house lysis buffer [2% SDS, 10mM EDTA, 10mM Tris-Cl, 50mM NaCl] with Proteinase K and limiting submersion time to thirty minutes for unfired and spent ammunition. This optimised method resulted in interpretable profiles for 26.1% of requested casework evidence samples. In a recent study simulating deposition of DNA via touch, Booth and Chapman [89] loaded serially diluted buccal suspensions from a volunteer on hollow point ammunition, fired and recovered DNA from bullets and respective fragments using a modification of the soaking method described by Dieltjes *et al.* [57]. While the concentration of recovered DNA showed a trend incumbent on the initial amount of cellular material deposited on the substrates, "repeatable partial profiles with five reportable loci pairs" that matched the donor's samples was only achieved in one undiluted replicate [89].

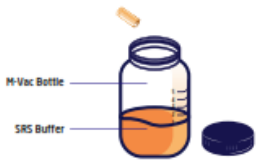
The soaking technique has been asserted to be more useful than just conventional swabbing of surfaces [90], but it suffers some critical limitations. Firstly, it is only suitable for samples within the size range of CBCs. Relatively bigger pieces of evidence (e.g. knife) will require an enormous amount of lysis buffer, extending processing times and complicating the extraction process due to the large volume of solution. Secondly, the submersion enhances the leaching of metal ions and contaminants, which are detrimental to nucleic acid integrity and adversely impact achieving interpretable profiles. Thirdly, as noted by Lawson *et al.* [87], the oxidation effects of lysis buffer on CBCs may cause the erosion of microscopic striations or riflings on casings that may have been useful to subsequent ballistic work. Finally, the destructive nature of submersion makes the technique unsuitable as a multi-stage investigation option. A typical multi-stage forensic analysis will entail, for instance, developing and examining fingerprints,

and sampling for DNA on a spent casing afterwards. Submersion in lysis buffer will destroy secretion (mainly amino acids, proteins, urea, lipids) etched into the metal surface, making subsequent fingermark enhancement infeasible.

3.4 Bardole MVAC method

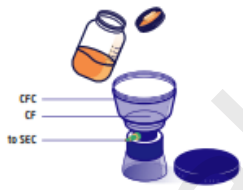
This technique was developed by Francine Bardole of West Jordan Utah Police Department with support of Microbial Vacuum Systems Incorporated (M-Vac Systems Inc). It is the most recent of methods aimed at enhancing nucleic acid recovery from problematic metal surfaces and has been acclaimed by some forensic scientists as "revolutionary" [91]. The M-Vac is a sterile-wet vacuum that loosens and sucks trace DNA evidence from samples that are difficult to swab for subsequent extraction [92,93]. The initial concept entailed washing down spent cartridge casings in a sterile buffer to cause skin cells to loosen into solution, followed by a filtering process that collects the cells for DNA extraction. The human skin sheds cells as part of a homeostatic regulation [94] and, at least, 500 million skin cells are lost per day [95,96] composed of fragmented or cell-free DNA enough to yield a genetic profile via PCR [52]. Spent casings typically have rough surfaces with many divots and grooves and microscopic crevices into which shed skin cells can embed, limiting the prospects of obtaining DNA evidence by swabbing from the surface. Bardole, utilising this prior knowledge and experience of working with an M-Vac, applied the this concept to a shell casing, which was the only evidence available in an unsolved case involving a random road-rage shooting incident [91]. The quantified extract yielded 0.847 ng of DNA and resulted in a full profile which matched the reference sample from a suspect, leading to a rightful conviction. In collaboration with M-Vac Systems, the "Bardole DNA Collection Method" was developed and is now a subject of scientific validation research [97]. A schematic of the technique is presented in Fig. 4.

The Bardole method is relatively simple, expeditious and does not cause leaching of metallic ions, which causes DNA damage, or erode ballistically vital rifling as in the soaking method. Furthermore, it increases DNA yield to the extent not possible with standard swabbing due to its ability to recover shed cells from small irregularities on the metal surface.



1. Add sterile collection solution (SRS buffer) to an M-Vac collection bottle and place evidence item (e.g spent cartridge casing (CBCs) in the bottle.

2. Agitate bottle to rinse cells from CBCs into solution



3. Attach Concentration Filter (CF) to the Support Equipment Case (SEC) vacuum hose and switch on the vacuum

4. Swirl and pour the solution from the M-Vac bottle into the concentration filter cup (CFC). Trap the evidence with the bottle cap to prevent falling onto the filter membrane (CF) and potentially damaging the integrity.

5. Allow to filter through the sterile 0.20 µm or 0.45 µm CF under vacuum pressure.

6. Rinse the bottle with SRS buffer or filtrate and repeat step 4 and 5, to ensure maximum transfer of collected DNA to filter.

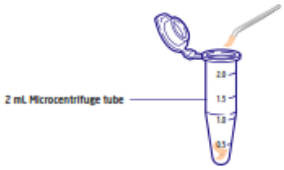
7. Filter membrane retains the DNA material

8. Turn off and disconnect vacuum tube when complete

9a. Cover CFC, label and allow CF to dry for 24 h in a clean environment. Cut out dry filter using sterile techniques.

9b. Alternatively, cut out filter material while wet if extraction is to be done immediately in a laboratory setting

10. Cut filter material with the retained DNA into pieces using sterile techniques.



11. Place samples into 2 mL microcentrifuge tube

12. Extract DNA from filter using standard protocol

Figure 4: **The Bardole M-Vac Method**. Schematic representation of the Bardole DNA collection method

3.5 Direct PCR

Direct polymerase chain reaction (direct PCR) is a sample processing technique proposed to circumvent DNA loss from trace sample during DNA extraction [98]. In the direct PCR process a sample (from standard swabs or a small piece of the substrate) is directly introduced into an amplification reaction without DNA extraction, quantification and purification steps [58,98,99].

The advocacy for the use of direct PCR has gained traction in recent times owing to advancements in touch DNA analysis, and the increasing tendency for touch DNA evidence to be submitted to forensic laboratories for examination [98]. The quest to limit processing time to potentially cater for casework backlogs and the knowledge that standard DNA extraction methods can cause an estimated 20% to 90% loss of initial template amount due to multiple wash steps and tube changes [100,101], make direct sample amplification attractive. Linacre *et al*. [102], as well as Vandewoestyne *et al*. [103], questioned the basis of touch DNA sample extraction given their already minuscule amounts and propensity for sample loss through the extraction process. Vandewoestyne *et al*. [103] demonstrated that cell-free DNA, which is a constituent of touch samples, was frequently lost through extraction and could be detected in 90% of supernatants of biological samples assessed. Hence, the inclusion of the retained cell-free DNA constituent of touched substrates in sample processing was mooted by Quinones and Danie [52] as a measure to maximise touch DNA typing, and this could be achieved through the exclusion of the extraction step, the fulcrum of the direct PCR method.

Templeton *et al.* [104] in a mock study, evaluated the utility of direct PCR on some surfaces including metals (brass, nickel and aluminium cartridge casings) through volunteer handling of uncleansed surfaces for approximately 15 seconds to facilitate fingermarks deposition. Sampling was performed after 24 hours and eight days via targeted swabbing [105], - in the case of the metals – subsequent to direct PCR using NGM™ kit. A 54% overall successful DNA recovery was realised, with highest from glass surface but none from the brass casing. Though the authors observed mixed DNA profiles, the major informative ones always matched the donor. The method has also been used to generate full genetic profiles from single hair follicles [58], fingernails clippings [101], clothing fibres [106] and touch DNA from various sources [107].

The direct PCR sample processing approach has been deemed a feasible alternative for forensic trace human DNA recovery and analysis, with attendant improvements in efficiency, sensitivity, as well as the quality of results [58]. Despite the above mentioned merits; extensive use in other fields [108–110]; potential for diverse applications in the forensic and investigative sciences domain - especially for metal exhibits that rarely yield informative DNA profiles (low copy DNA samples) [107]; - and development of commercial products tailored for its application [98,111], the method is as yet not widely used in an operational sense in most forensic laboratories. The problem in operationalising the direct amplification approach is primarily related to:

1. PCR inhibition which ensues once substances interact with the polymerase enzyme, the DNA molecule or cofactors necessary for polymerase function, thus, preventing either partial or full amplification of DNA [99,112] and
2. The total lack of the possibility to perform any repeat measurements (re-testing) from the same sample.

Metals encountered in crime scenes may habour other trace biological matrices together with deposits from the touch, on their surfaces. These biomaterials may be potential sources of inhibitors when swabbed and directly introduced into a PCR reaction, and may include humic acid from soil/settled dust particles [113,114]; haematin and other compounds contained in trace bloodstains [115,116]; metal ions, notably, in oxidized state [49] as well as other environmental contaminants. McCord *et al*. [117] found that, for an inhibited DNA sample,

505 there was a steady loss of larger amplicons in STR analysis with increasing inhibitor
506 concentrations. However, the influence of inhibitors on PCR has been minimised due to
507 advancements in polymerase buffer technology [118]. A potential inhibition source that has
508 been overlooked in the move towards direct PCR for trace DNA work is the sampling devices –
509 the swabs. The presence of metal-derived ions and other contaminants within the commercial
510 swabs has not been investigated. The presence of inhibitors going straight into the PCR is
511 undesirable and will impact on the uptake of this method.

512

513

514 **4. Effect of substrate surface**

515 The surface characteristics of a substrate are relevant to nucleic acid persistence and recovery.
516 For example, roughness (compared to smooth surfaces) was linked to an increase in recovery of
517 bacterial spores from different spacecraft-related surfaces, using nylon-flocked swabs [119];
518 and a parallel observation regarding efficient trace DNA recovery was made for ridged bottle
519 tops [61]. A study examining fired weapons observed higher success rates of recovery from
520 rough and textured surfaces of handguns than smoother surfaces [120]. However, as noted by
521 Verdon et al. [70], some swabs materials may be left on rough-textured surfaces limiting
522 sample collection capacity, and the loose fibres, when retained in a reaction mixture, could
523 result in PCR inhibition [54].

524 Touch DNA on guns may be degraded by the percussive shock and high temperatures
525 generated during firing, as well as by interaction with other substances such as unburned
526 gunpowder, gun lubricant and gunshot residues [57,121]. Despite this Fan et al. [120]
527 demonstrated the ability to recover touch DNA from different parts of fired guns and CBCs.

528 The abrasive nature of rough-textured substrates surfaces such as slide serrations, grip panel
529 and magazine releases of handguns enhances epithelial cell shedding during the process of
530 handling a firearm and may facilitate the accumulation and retention of cellular material [61].
531 Notwithstanding, the available studies utilising various metallic materials including firearms and

CBCs (for example [13,122,123] or exploded improvised explosive devices (IEDs) [123–126] have focused on method validation (i.e. extraction efficiency of various reagents, buffers, swabs, and protocols) and provide no further insight on the relevance of specific substrate or surface conditions (such as metal type and alloy composition, surface cleanliness and/or extent of rust, gross/microscopic surface topography) and their effect on recovery and subsequent downstream forensic processes. Further, sample collection (mostly swabbing) in these works are undertaken almost immediately following touch sample deposition or within 24 hr, presenting a difficulty in establishing the influence of the 'touch interval' (the time elapsed since the initial touch sample deposition) on sampling and recovery efficiency. Broader research, employing larger sample sizes with different ranges of bio-analytical experimental approach to the existing research, is required to address the enumerated problems to inform frontline forensic practice.

5. Future directions

Extensive research is needed to enhance understanding of metal-DNA interactions in the context of forensic investigations. This should include a systematic study to evaluate the effect of conditions including alloy composition, surface texture, extent of rust and the effect of environmental exposure on persistence, recovery and amplification of trace DNA samples.This will inform better sample collection, extraction and clean-up to improve profiling of DNA recovered from metal surfaces. Testing across a range of metals will also enable the triage of metal exhibits, facilitate cost-effectiveness and fast analytical throughput. While consistent development and validation of new methods and refinement of existing techniques should ultimately culminate in improvements, it is instructive that the standard swabbing methods, along with direct PCR, have the highest prospects owing, especially to the relative cheap cost and ease of training needs. Research is thus required to explore the possibility of metal-derived contaminants/inhibitors inherent in the swab devices (from manufacture) and to examine their impact on recovery and downstream processes.

6. Conclusions

Understanding metal-DNA interactions, including the impact of specific metal composition and surface conditions on DNA recovery, are fundamental to improving the chances of obtaining interpretable profiles from trace sample sources. This review has highlighted the current scope of research, enumerated some limitations and suggested further research directions to address them. Such investigations will enhance the forensic capabilities of law enforcement in general and benefit crime laboratories during investigations by improving the prospects of producing interpretable DNA profiles, especially in situations where there is a lack of other probative evidence.

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7. Acknowledgement

Dan OM Bonsu is financially supported by the Adelaide Scholarship International (ASI) and the Bill Retalic Prize for Science and Justice for his doctoral study at The University of Adelaide, Adelaide, Australia. We thank Dr. Jennifer Raymond (Research Coordinator, Forensic Evidence & Technical Services Command, NSW Police Force) and Dr. Julianne Henry (Forensic Science South Australian (FSSA)) for their helpful comments.

Forensic touch DNA recovery from metal surfaces – a review

Highlights

- Metal surfaces are difficult substrates for trace DNA recovery and amplification.
- Metal cations interact with DNA via complex intercalation, irreversible covalent binding and groove association
- Five methods of touch DNA sampling include swabbing, tape lifting, soaking, Bardole MVAC and direct PCR
- There is at most 26% DNA recovery success rate from cartridges, bullets and casings (CBCs)
- The surface characteristics of metal substrates are relevant to nucleic acid persistence and recovery

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