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Effects of chemical & biological warfare agent decontaminants on trace survival: Impact on DNA profiling from blood and saliva

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ABSTRACT

Forensic investigations following incidents involving chemical or biological agents present considerable challenges. Understanding the possibilities and limitations can aid in determining the most suitable procedures and enhancing the recovery of useful traces in these complex situations. This work complements previously published results on the effects of decontaminants on fingermarks deposited on glass. Identifying the perpetrators can be crucial, and DNA analysis remains a cornerstone in this regard. In this study, we investigated the ability to obtain usable DNA profiles from blood and saliva (pure and diluted) exposed to 16 different decontamination methods. Both DNA quantitation and DNA profiling were considered to assess the outcomes. The results revealed considerable variability but indicated that biological agents' decontaminants hindered DNA profiling post-decontamination to a greater extent than decontaminants aimed for chemical agents. Chlorine-based decontaminants also globally had a deleterious impact on DNA profiling. Powder decontaminants such as Fast-Act, CHpowder, and the liquid decontaminants GDS2000 did not affect DNA profiling.

1. Introduction

Although such incidents are thankfully rare, the process of conducting forensic investigations subsequent to events involving chemical or biological agents presents significant hurdles and complexities. One such hurdle is deciding the overall strategy of forensic items and traces processing. Should objects be examined in safe conditions, i.e. in a dedicated laboratory with suitable facilities and equipment, or should they be decontaminated before forensic examination? The first scenario involves the implementation of adapted approaches and methods which can be applied within the constraints of the infrastructure. The second scenario allows for the ideal application of techniques but carries the risk that traces may be compromised by the decontamination procedure involved [1,2]. Consequently, it is important for forensic science to explore its interface with CBRN (Chemical, Biological, Radiological and Nuclear) fields. This entails developing an understanding of the impact of agents and procedure on the traces, which in turn allows for the decision-making process regarding which objects and traces to collect. This work complements previously published results on the effects of decontaminants on fingermarks deposited on glass [3].

Within the array of traces that can be encountered during forensic investigations, DNA has become a valuable and indispensable component across various case types [4,5]. DNA unveils a paradoxical nature, it can be easily removed or damaged by different factors [6–10], yet only few cells can provide sufficient DNA for a profile [11,12]. DNA is susceptible to degradation from environmental factors such as light, heat and humidity [9]. Exposure to UV light can damage DNA, elevated temperatures can lead to denaturation and fragmentation and high humidity levels can contribute to the degradation through hydrolysis reactions, leading to the loss of nucleotide bases, deamination, and strand cleavage of DNA [8,13]. Common degradation mechanisms are hydrolysis and oxidation, in particular reactive oxygen species and free radicals, specifically hydroxyl radicals, are well known to cause DNA degradation [8,14,15]. Additional factors such as extreme pH levels and microbial activity can further contribute to the fragmentation and modification of DNA profiles [6,8,16].

Despite these challenges, the possibility of recovering DNA profiles from crime scenes and from items exposed to extreme temperatures (e.g. arson or IED incidents)[17–20] or high humidity (submerged items) [21–24] has been demonstrated to be feasible.

A number of fundamental studies have examined the ability to recover DNA profiles following the exposure to various cleaning agents

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[5,25–31], biological decontamination means [32–34], or the exposure to biological and chemical agents [33,35–37]. The outcomes of these studies are already of some use to the CBRN field.

The impact of cleaning agents on DNA recovery reveals a variable landscape. Substrate, DNA carrier and sampling methods, along with analytical approaches, introduce considerable variations in both DNA quantity and ability to recover profiles [25–29]. Commonly investigated cleaning agents are soap and bleach [30,31]. Overall porous substrates have been shown to allow for higher success rates for DNA profile recovery. Chlorinated cleaning agents, like bleach, have shown a high tendency to degrade DNA, resulting in lower profile recovery rates [5].

In their 2020 study, Wilkinson et al. investigated the impact of various liquid and gaseous biological decontamination methods on DNA profile recovery. Notably, formaldehyde and ozone significantly affected DNA quantity, preventing full profile recovery. Assessing both quantity and quality, Dry fogging, MODEC MDF-500, and Bioxy-S were identified as the least damaging decontamination agents. Additionally, this study highlighted vaporized hydrogen peroxide and gamma irradiation as the least destructive methods for DNA profiling in the presence of biological agents [33].

Ionizing radiations are commonly used to neutralise biological agents [38]. The impact of various radiation on DNA profiling, such as alpha, beta, and gamma irradiation, as well as UV irradiation, has yielded diverse outcomes [34,39,40]. Factors such as the applied dose, the specific DNA analysis protocol, and the substrate and DNA carrier in presence, contribute to variable results. Overall, while these radiations effectively damage biological agents [41], their impact on human DNA and DNA profiling is equally acknowledged, although to a variable degree [39,40].

Concerning biological or chemical warfare agents, Timbers et al. [36] investigated how biological hazards, such as bacteria, endospores, toxins, and viruses persist during DNA extraction process for genetic profiling. Their results indicated that bacteria, viruses and toxins were decreased to undetectable levels during the extraction through the action of the lysis buffer. However, viable endospores persisted after extraction. Implementing a filtration step post-DNA isolation successfully eliminated viable spores without compromising the human DNA. The study noted that prolonged contamination of samples with certain biological agents hindered the ability to complete genetic profiling, emphasizing the importance of prompt and effective decontamination measures [36].

Chemical warfare agents present an intricate interference canvas when it comes to DNA. Wilkinson et al. [35] have demonstrated that certain agents, such as hydrogen cyanide, sarin, sodium fluoroacetate and diazinon, do not inhibit DNA profiling. However, the alkylating nature of other agents like sulphur mustard and dimethylsuphate, and the oxidative nature of others, such as chlorine and phosgene may not only degrade DNA but can even lead to the complete loss of the genetic material [35].

A nuanced understanding of how DNA traces persist in various scenarios can significantly enhance investigators' abilities to target locations more effectively, ultimately optimising efficiency and minimising both costs and time investments [4,42]. Our study aims to complete and expand the knowledge on trace survival in specific conditions, in particular when exposed to chemical decontamination methods, and help increase preparedness for CBRN mitigation strategies, aligning with the recommendations of Socratous and Graham [43].

In the present study, 16 different decontamination means have been applied on both blood and saliva samples. The blood and saliva samples have been deposited on gauze then exposed to the decontaminants. Subsequently, DNA extraction, quantification and profiling have been completed, providing an indication of the impact of the decontamination procedure on DNA profiles from such biological traces. Decontaminants included commonly available cleaning agents as soap, water, and bleach as well as specific decontaminants specifically developed for first responders and CBRN mitigation teams to target chemical and biological warfare agents.

2. Materials and methods

2.1. Sample preparation

Two body fluids were selected as carriers of DNA: whole human blood and human saliva. These were chosen as they typically contain a high amount of DNA [44] and generally enable more reproducibility than touch DNA for research purposes [45]. The study was performed using both pure and twofold diluted blood, as well as pure and twofold diluted saliva.

The study employed undiluted human blood treated with EDTA, obtained from a single donor through the blood bank. The blood was stored in the refrigerator at 4°C. A twofold dilution of blood was prepared with half volume of the blood and half volume of sterile water. The diluted blood was equally stored in a refrigerator at 4°C between use.

For the saliva, a single donation of saliva was requested from a human donor. The donor was instructed to fill a sterile 15 ml tube between 8 and 9 a.m. before having breakfast [46]. The saliva was stored in a freezer at −80°C to avoid bacterial degradation of the DNA and enhance DNA conservation [47]. For the preparation of the diluted saliva samples, a twofold dilution of saliva was freshly prepared prior to each use with half volume of saliva and half volume of sterile water.

All the body fluids were set to room temperature and vortexed thoroughly before use. After use, the body fluids were returned to the aforementioned (fridge or freezer) storage condition.

10 µL of undiluted and diluted blood and 40 µL of undiluted and diluted saliva were spiked onto the estimated centre of squares of three-layered sterile gauze (Toppers™ 12, Systagenix), with dimensions of approximately 1.5 cm square. They were left to dry overnight at room temperature in a DNA hood. For each sample, the gauze squares were placed separately into previously cleaned glass Petri dishes. The gauze squares and the Petri dishes were previously exposed 30 min to UV light (254 nm) to further prevent DNA pollution remaining on the gauze or the Petri dishes.

2.2. Decontamination

The decontaminants have been selected to represent a large spectrum of active compounds (Table 1). The liquid decontaminants (aqueous and organic solvents) were applied in accordance with manufacturer's instructions (Table 1), either by immersing the samples or by spraying them. For the immersion, 15 ml of decontaminant was poured onto the gauzes in each Petri dish. For decontaminants that were sprayed, the gauzes were completely covered and sprayed onto until completely soaked. The powders and the RSDL were poured onto the gauzes until completely covering them.

The gauzes were subjected to various durations of exposure to the decontaminants in accordance with manufacturer's instructions (Table 1). The decontamination operations were conducted at room temperature. After application of the decontaminants, the gauzes were removed and placed in clean petri dishes using sterilized tweezers, and no action was undertaken to remove excess decontaminants from the gauzes. They were left to dry overnight in a closed DNA hood to prevent contamination.

The procedure was replicated three times for each decontaminant and each body fluid combination. Three blood and saliva samples were equally deposited on gauzes for each concentration without undergoing any treatment to establish reference results for each. One blank sample (i.e. a gauze without any DNA) was decontaminated with each decontaminant to ascertain that the decontaminants do not contain any DNA that may pollute the samples.

Table 1
Description of the decontaminants and their applications based on the manufacturer's instructions.

Main Target	Main chemical mechanism	Decontaminants *(Manufacturer/ Supplier)*	Actives substances	Application	Exposure time
Chemical agents	**Physical Removal**	**Water*** *(from the tap)*		Immersion	5 min
		Soap Water* **~ 0.1 % (v/v)** *(Tap water with "Handy" detergent[a])*		Immersion	5 min
		Isopropanol* (*Sigma-Aldrich >99.8 %*)		Immersion	5 min
		SkinNeutrAll®* *(Ilma Biochem)*	Ascorbic acid	Spray	5 min
	Absorption Adsorption Oxidation	**CHpowder** *(Swiss Armed Forces Command Support Organisation)*	Chlorinated lime (Calcium hypochlorite), Magnesium oxide	Powder	90 s
		FastAct® *(Enware)*	Magnesium oxide, Titanium oxide	Powder	90 s
	Nucleophilic substitution	**GDS2000** *(Kärcher)*	2-Aminoethanol, Diethylentriamine	Spray	10 min
		GD6 *(OWR)*	Aminoethanolat	Spray	15 min
		RSDL® *(Emergent Bio)*	2,3-butanedione monoxime	Recovery[b]	2 min
	Oxidation	**Alldecont** *(OWR)*	Sodium hypochlorite	Immersion	2 min
		BX24 ~ 10 % (w/v) *(Cristianini)*	Dichlorisocyanurate	Immersion	15 min
		Commercial Bleach *(Potz)*	Sodium hypochlorite	Immersion	5 min
		Wasa®-Soft & Clorina® ~2.5 % (v/v & w/v) *(Lysoform)*	Tosylchloramide	Immersion	2 min
Biological agents	**Oxidation**	**Virkon®S ~1 % (w/v)** *(Lanxess)*	Pentapotassium bis(peroxymonosulphate) bis(sulphate), Benzenesulfonic acid	Immersion	10 min
		Vaprox diluted ~10 % (v/v) *(Steris)*	Hydrogen peroxide	Immersion	15 min
		Wofasteril ~2 % (v/v) *(Kesla)*	Peracetic acid	Spray	60 min

*Investigated as removal method, but with hydrolysis capabilities.
a Handy" is a Swiss dish soap detergent brand manufacturer by Migros
b Recovered with the substance

2.3. DNA analysis

The DNA analysis proceeded through a series of steps: extraction, quantification, amplification, and sequencing. However, two specific stages were particularly significant in this study (Fig. 1). Firstly, quantification data were gathered to investigate the impact of decontaminants on the DNA quantity that could be recovered from these samples. These data then guided further sample preparation, determining the required extract amount for amplification. Subsequently, amplicons underwent analysis via capillary electrophoresis, with the assessment of Short Tandem Repetition (STR) profile recovery conducted through the combined analysis of each duplicate.

2.3.1. Extraction

DNA was extracted from each gauze through the combined use of the Investigator Lyse&Spin Basket Kit (Qiagen AG) and QIAamp DNA Mini Kit (Qiagen AG) following a procedure established by a forensic genetics laboratory accredited to ISO 17025 standard [48]. The gauzes were placed in spin basket tubes. 180 µl of ATL buffer and 20 µl of proteinase K were added to each tube. The tubes were then vortexed and incubated for 1 h at 56 °C at 500 rpm on a Thermoshaker (BioSan, TS-100). The spin basket tubes were finally centrifugated 5 min at 12000 rpm. The spin basket and the gauzes were then removed, and the DNA was purified following the procedure provided by the QIAamp DNA Mini Kit (Qiagen AG)[49].

Positive controls (swab with 50 µl of 1/250 diluted blood from a known donor) and negative controls (empty spin basket) were added to each extraction sessions.

2.3.2. Quantification

DNA was quantified with the Quantifiler™ Trio DNA Quantification Kit (Applied Biosystems™ by Thermofisher Scientific) following manufacturer's instructions. The quantification was done using a QuantStudio™ 5 real-Time PCR System (Applied Biosystems™ - Thermofisher) and the associated HID Real-Time PCR Analysis Software. Each extraction sample was quantified twice, as shown on Fig. 1.

2.3.3. Amplification

The AmpFLSTR NGM SElect PCR amplification kit (Applied Biosystems™ by LifeTechnologies – Thermofisher scientific) was used for the DNA amplification on a ProFlex™ 3 ×32-well PCR System (Applied Biosystems™ by LifeTechnologies – Thermofisher scientific). The chosen kit targets 16 STR (Short Tandem Repeat) loci and amelogenin using 5 µl of the DNA extract in a 25 µl total PCR volume when its

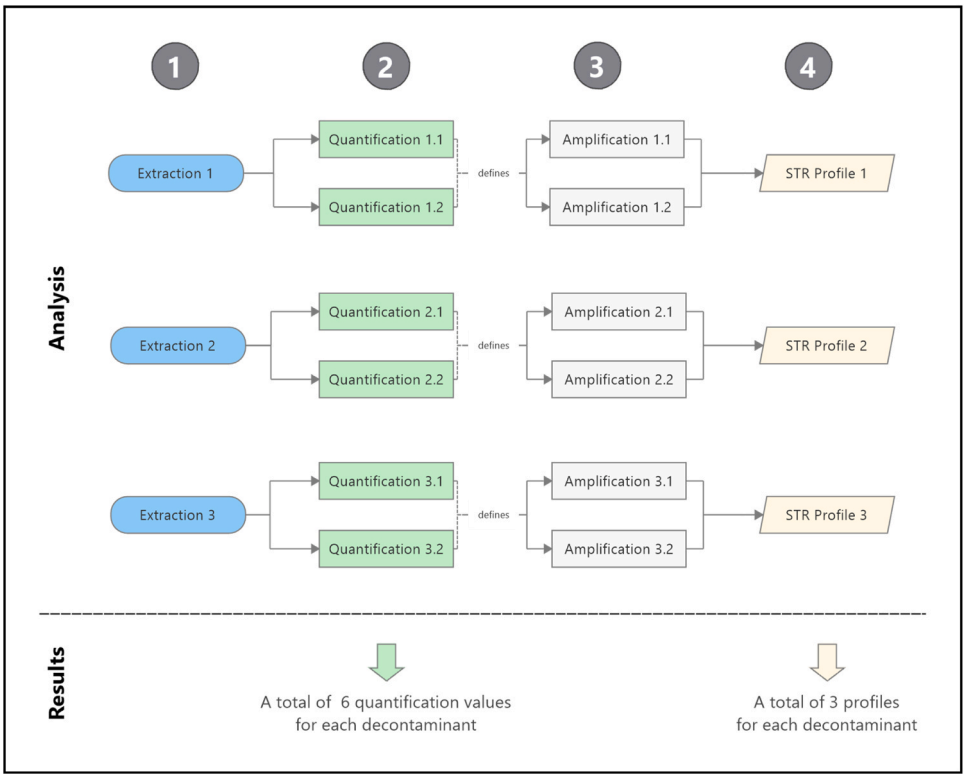

Fig. 1. DNA data collection process for each decontaminant and each sample dilution.

concentration was less than 0.1 ng/µl. When the concentration was higher, the volume of DNA extract added to the PCR volume was reduced. Above 0.75 ng/µl, samples were dilute to obtain a 0.5 ng/µl concentrated extract. The DNA from each extraction was amplified twice using the same conditions (two replicates per sample, Fig. 1). Positive and negative controls were added to each amplification bloc.

The thermal cycling program involved an initial denaturation at 95°C for 11 minutes, followed by 30 cycles of denaturation at 94°C for 20 seconds and annealing at 59°C for three minutes. The process concluded with an extension of the loci at 60°C for 10 minutes. After completion, reaction vials were temporarily stored in the thermal cycler, maintained at 4°C indefinitely prior to being transferred to a freezer at −20°C.

2.3.4. *Sequencing*

Samples were prepared for capillary electrophoresis analysis by dispensing 9.5 µl of Hi-Di™ formamide (Applied Biosystems™) and 0.5 µl of GeneScan-600 LIZ™ (Applied Biosystems™ by Life-Technologies – Thermofisher Scientific) into each well, and 1 µl of each amplified sample was added. Per 96-well plate, twelve allelic ladders standards were added at the bottom of each column. Following sample transfers, plates were covered with septa pads and directly loaded onto the 3500 Genetic Analyser for sequencing. The amplified DNA was analysed with the 3500 Genetic Analyser (Applied Biosystems™ by LifeTechnologies – Thermofisher Scientific) following standard procedures.

2.4. *Data processing*

Quantification data was analysed using the integrated tools provided by QuantStudio™ Design and Analysis desktop software, assessing correct amplification through visualisation of the amplification plot and standard curve based on reference values. Quantification values and Degradation Index (DI) were retrieved in Excel.

Quantitative results were first study by themselves then put into perspective with the Degradation Index provided by the Quantifiler™ Trio DNA Quantification Kit. This DI estimates DNA degradation, through the fragmentation based on the ratio between the concentration of small and large autosomal amplicons (i.e. in the case of the Trio quantification kit, respectively an autosomal target of 80 bp and a "degradation target" of 214 bp) [50]. DI values below the manufacturer's threshold of 1.5–2, indicate no degradation of DNA and DI values above 10 indicate that the DNA is severely degraded [50,51].

Following QuantStudio™ user manual [51] and drawing from previous research by Vernarecci et al.[52], it has been demonstrated that combining the quantitation value with the DI can offer a reliable prediction of the chances of profile recovery. In scenarios involving CBRN and large numbers of samples, the combination of quantitation values with DI can provide central information for triage and help determine sample which should be analysed first. Given the expected degradation and time sensitivity that may be important in such cases, exploring this approach seemed appealing given the studied context. Based on Vernarecci et al. prediction process (Fig. 2) [52], combined with the evaluation criteria set in Table 2, it was possible to evaluate the possibility to anticipate the chance for complete profile recovery considering quantity and DI.

The GeneMapper ID-X software (v1.4, Life Technologies) was used to process the DNA profile data. Analytical thresholds for profile data interpretation were set based on protocols applied by a Swiss forensic genetics accredited laboratory for traces in real case work. Analytical thresholds in GeneMapper were set to 70 RFUs (Relative Fluorescence Units) for the blue, yellow and green fluorophore and of 100 RFUs for the red fluorophore.

Following the software's assignment of allele, the identification and rectification of any artifacts, including pull-up, drop-in and drop-outs were carried out in accordance with the protocols provided. For each extraction, a DNA profile is determined to possess an allele if in both analyses the allele is correctly detected and attributed. For the final evaluation three categories were determined: complete profile, partial profile and no profile following the criteria in Table 2. The criteria were

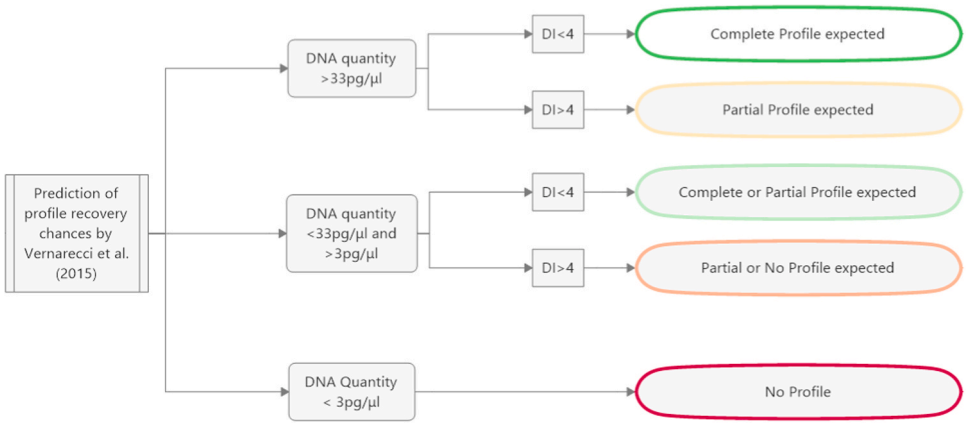

Fig. 2. Profile recovery prediction tree based on published data by Vernarecci et al. [52].

Table 2
Evaluation categories for DNA profiles applied in our work based on [21].

Categories	Description
Complete Profile	If all 16 loci and the amelogenin marker had every allele of the individual in question, the result was considered a complete profile.
Partial Profile	If at least 6 loci had every allele of the individual in question, the result was considered a partial profile.
No Profile	If fewer than 6 loci had every allele of the individual in question, the result was considered as no profile.

determined based on interpretation guidelines provided by a Swiss accredited forensic genetics laboratory, and similarly to the approach chosen by Helmus et al. [21]. Complete and partial profile both meet the requirements for a single profile to be sent to the Swiss CODIS Database.

Additionally, profile shape was observed, and elements of DNA degradation were noted. Specifically, profiles were examined to identify the presence of a "ski slope effect." This effect can be visualized through high peak heights in short amplicons and low peak heights in long amplicons on the electropherogram and indicate DNA strand fragmentation and overall DNA degradation [13,50,53].

No statistical evaluation was performed due to the limited samples size for each subset and a descriptive approach was preferred.

3. Results

3.1. Quality control and blank samples

All positive and negative extraction controls and all positive and negative amplification controls yielded expected results (no alleles in the negative controls, complete and concordant profiles for the extraction and amplification positive controls), indicating successful completion of all steps and allowing to certify successful extraction, amplification, and capillary electrophoresis analysis.

For the quantification, the standards for the calibration curve met the expected values provided by the manufacturer [51].

References samples yielded full profiles and were used to assess the profile recovery for each decontaminant.

The blank sample treated solely with decontaminants showed no presence of DNA, and consequently, no profile could be extracted from it, indicating no DNA pollution (Supplementary material - Image 1).

3.2. Visual samples examination

The red colour of blood provided a first visual indication of the effects of the decontaminants (Table 3). It's important to clarify that while these observations suggest a reaction, it doesn't necessarily indicate on their impact on the DNA present in the blood. However, visual examination contributes to a better understanding of the mechanisms involved.

Table 3
Visual observation of the gauze and the reaction of the blood and the decontaminant.

Decontaminants	Modification of the appearance of blood on the gauze
Water	Blood partially dissolved/wash out
Soap Water	Blood partially dissolved/wash out
Isopropanol	No change observed
SkinNeutrAll®	No change observed
CHpowder	White coating on blood due to powder residue
FastAct®	White coating on blood due to powder residue
GDS2000	No change observed
GD6	Blood takes a pink coloration and is fading away
RSDL®	Blood takes a darker color
Alldecont	Blood color changes to brown
BX24	Blood color changes to brown
Commercial Bleach	Blood color changes to brown/green
Wasa®-Soft & Clorina®	Blood partially dissolved and blood color changes to brown
Virkon®S	Blood color changes to yellow
Vaprox	Creation of foam and no visual blood stain remained
Wofasteril	Blood color changes to yellow

In the cases of water and soapy water, the observation of dilution and dissolution of blood from the gauze suggests a washing-off effect. The change in color from red to brown, for Bleach, Alldecont, BX24, and WasaSoft & Clorina could illustrate the preferred oxidative action on iron-containing molecules [54], and could indicate the accelerate oxidative action on hemoglobin and its conversion to methemoglobin which has a dark red-brown coloration [55]. DNA present could also be affected [56,57].

Vaprox, containing hydrogen peroxide, resulted in a foaming reaction, mostly documented as the reaction between the blood's catalase enzyme which leads to the release of oxygen [58,59]. After exposure to Vaprox, no visible blood remained on the gauze hinting on possible DNA loss [60]. Peroxides typically react through the so-called Fenton reaction with iron species present in the blood [61]. This reaction pathway could contribute to an oxidative process leading to a yellowish hue. Additionally, hydrogen peroxide could lead to the Haber-Weiss reaction mechanism with iron ions, which would create hydroxyl radicals. These radicals are highly reactive and have strong abilities to degrade DNA [62].

For Wofasteril and Virkon, the change to a yellowish color/fading away was observed, suggesting their oxidative action on blood although detailed mechanisms cannot be provided. Similarly to Vaprox, DNA loss with these decontaminants would be expected.

GD6 induced a pink coloration of the blood spots, the underlying

chemistry remains unclear, but iron-binding effect could be expected. Powders (FastAct and CHpowder), isopropanol, GDS2000 and SkinNeutrAll did not visually affect the blood stains.

These visual observations of color changes help indicate reactions between the blood and the decontaminants. However, the complex mixture of blood precludes an extrapolation of the impact of these decontaminants on the DNA contained within the blood. It is evident that the reaction mechanisms can be diverse, emphasizing the need to investigate how these various decontamination methods affect, if they do, forensic DNA profiling.

3.3. Quantification

The total DNA quantity recovered from undiluted and diluted blood varied greatly, ranging from 0 to 8000 pg/µl, depending on the decontaminant used (Fig. 3). For undiluted and diluted saliva, the recovered DNA quantities were found to be lower overall, with the highest concentration just below 3000 pg/µl and some samples showing no detectable DNA. In comparison to blood samples, saliva samples exhibited less variability in quantification and generally had lower DNA concentrations. However, variations were still noticeable between the three different extractions. Quantification replicates within the same extraction tended to show less variation for saliva than for blood where up to 85 % relative standard deviation (RSD) between replicas of the same extraction was observed.

In undiluted blood samples, only four decontaminants (RSDL, SkinNeutrAll, BX24, and Virkon) yielded more than two replicates with quantitative results below 30 pg/µl, indicating good DNA quantity recovery after treatment with almost all the decontaminants. In the diluted blood samples, DNA was not detected in any of the replicates for BX24, Bleach, and Virkon.

In undiluted saliva samples, no DNA was recovered in any of the six replicates for bleach and Virkon, and for BX24 and Vaprox, only very low DNA concentrations (between 0 and 14 pg/µl) were observed. For diluted saliva samples, in addition to bleach and Virkon, BX24 yielded no recoverable DNA, while Alldecont and Vaprox had low DNA concentrations (below 30 pg/µl).

Blood and saliva are complex fluids and although blood yielded overall high amounts of DNA, its composition also includes various proteins and elements that may interfere during the extraction and quantification process, contributing to the higher variability within the quantitative DNA results. Preliminary tests (results not shown) also indicated that the extraction process may induce high variation [11], as do the substrate on which the DNA is extracted from. First tests were performed with blood deposited on 100 % cotton paper as tested by Wilkinson et al. [33]. However, the extraction efficacy was found to be low, and thus gauze was employed as an alternative. This further emphasise the crucial impact of the substrate on success in real cases, as mentioned by Sewell et al. [63].

Therefore, the observed intravariability can be traced back to the initial differences in DNA quantity from the sample on the gauze, the extraction procedure, and the quantification process itself. This is illustrated by the intravariability seen within the reference samples, which have not undergone any decontamination process yet still exhibit considerable variability. Nonetheless, high recovery amounts of DNA were consistently recovered. Navigating this variability is essential to discern which aspects cannot be ascribed to the typical variability of these processes.

Decontaminants that yielded low quantities of DNA are particularly valuable for clear interpretation, as they provide evidence of a deleterious impact of the decontaminant on DNA recovery that cannot be accounted for by the intrasample variation or the DNA extraction and quantification method.

Based on quantitative results of all four sample types tested, Virkon had the most significant negative impact since almost no DNA was recovered for either blood nor saliva. BX24 and bleach were also strongly detrimental with only minimal amount of DNA recovered from the undiluted blood and saliva, and no DNA from the diluted samples.

For Vaprox, relatively high amounts of DNA were recovered from undiluted blood, but the quantities were considerably lower for diluted blood and the saliva samples.

Isopropanol and WasaSoft/Chlorina and Wofasteril had overall high quantification results but also had high variation within the different extraction, suggesting an impact on DNA, albeit in a less pronounced and less repeatable manner.

Within the other decontaminants (i.e. soap, GD6, RSDL, SkinNeutrAll and Alldecont), the variation and lower DNA quantity values suggested that some did degrade or remove DNA, although not necessarily

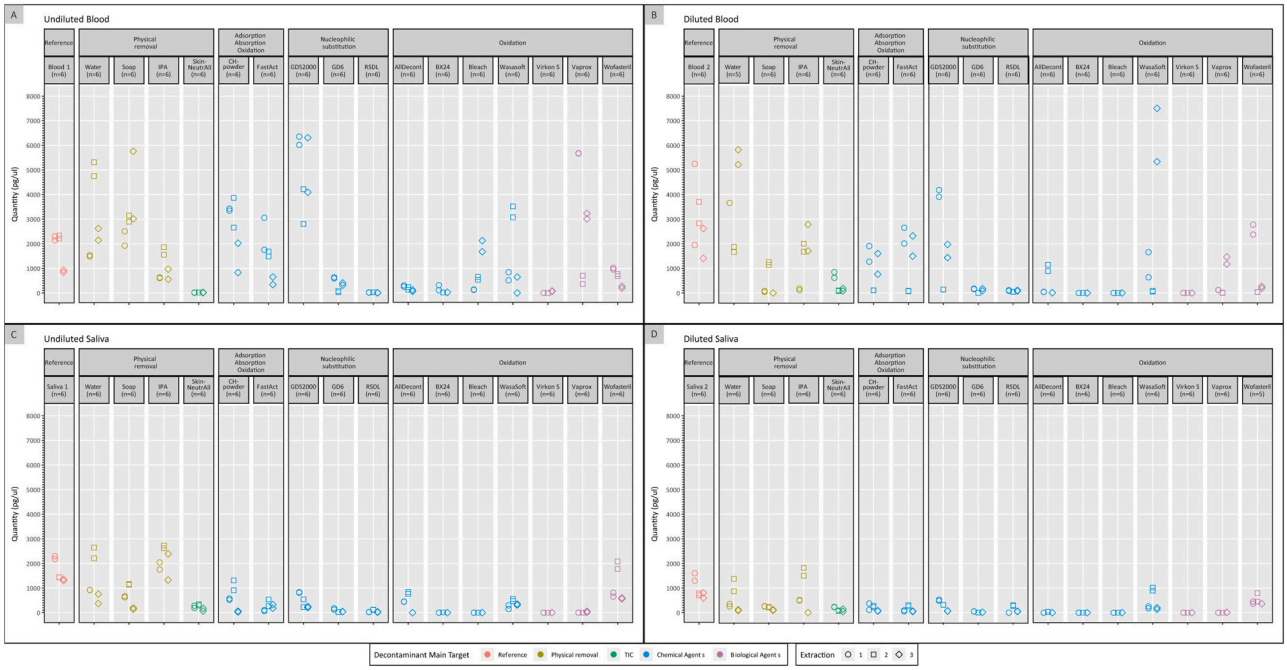

Fig. 3. Scatterplot of DNA concentration for each sample type.

enough to establish a clear trend.

Water, GDS2000, as well as the CHPowder and FastAct, did not seem to have a direct impact on the quantity of available DNA material when applied on blood.

To further explore these results, the DI values were utilised. The values for the saliva samples were mostly ranging between 1 and 3, compared to the blood samples for which most DIs were below 1 (values can be seen on the barplot in Fig. 4). Given saliva's composition with numerous enzymes known to degrade DNA, this outcome is not unexpected [64].

The samples that showed high DI values (> 4) were samples decontaminated with GD6, SkinNeutrAll, RSDL, Vaprox, Virkon and Wofasteril. Vaprox, Virkon, and Wofasteril are decontaminants designed to be efficient against biological agents and are commonly employed to combat pathogens and disinfect surfaces [65–67]. It's not surprising that they exert a similar decontamination mechanism on human cells as they do on microorganisms, leading to DNA degradation.

Using the decision tree (Fig. 2) and the threshold value of 4 (to take into consideration the increase degradation of saliva samples), only 49 out of 384 quantified samples had a DI value above 4. The majority of these samples were the ones decontaminated with Wofasteril (Fig. 4).

RSDL, GD6 and SKinNeutrAll were notable because while varying amount of DNA could be recovered by the samples decontaminated with these substances, their DI suggests that some DNA degradation occured and as a result, partial profiles could be anticipated. Applying the aforementioned Vernareccis' decision criteria, the STR profiling following these decontaminants should allow only for partial profile recovery which was the case vide infra. Combining quantity and DNA degradation index and applying Vernarecci's approach, the prediction for full, partial, or no profile aligned in 93 % of the cases with the obtained profile results.

3.4. DNA profile recovery

For undiluted blood, one decontaminant (Virkon) yielded no profile, three (SkinNeutrAll, Vaprox, and Wofasteril) resulted in partial profiles, while the 12 others gave complete profiles (Fig. 4).

For the diluted blood samples, similar trends were observed, with two additional decontaminants, BX24 and Bleach, resulting in no profile. The second extraction of the undiluted saliva sample for BX24 resulted in a profile with several alleles that could not be attributed to the donor. Interpretation of this profile was difficult, and to avoid false interpretation, it was not considered further (marked as NA in Fig. 4). The samples exposed to GD6 resulted in two partial profiles although only one locus was missing and in one extraction no DNA profile could be recovered. For one sample of diluted blood exposed to soap, no profile was recovered.

For undiluted saliva, 7 decontaminants gave complete profiles for all three extractions. CHPowder, FastAct, GDS2000, WasaSoft/Clorina and Water allowed for complete profile recovery for both undiluted and diluted saliva samples and showed overall no signs of DNA degradation.

BX24, Bleach and Virkon gave no profile neither for undiluted nor diluted saliva.

While one soap extraction yielded no profile for undiluted blood, a similar situation occurred for one extraction of diluted saliva exposed to isopropanol, where no DNA or DNA profile was recovered.

Similar trends were observed between undiluted and diluted saliva samples. GD6, SkinNeutrAll, and Wofasteril resulted in three partial profiles each. RSDL allowed for the recovery of two complete profile and one partial profile for both saliva concentrations. Alldecont yielded two complete profiles, although one extraction resulted in no profile for undiluted saliva, and only one complete profile for diluted saliva. Vaprox did not produce a profile for diluted saliva and only allowed for one partial profile recovery for undiluted saliva.

Examination of the affected markers for partial profiles revealed the

Fig. 4. Total Loci and profile recovery. The mean quantification and DI values for each extraction are written in the centre of the bar. Extractions that yielded a mean DI value above 4 are written in red.

symptomatic 'ski slope effect' on them. For undiluted blood, all three decontaminants showing partial profiles (i.e. Vaprox, Wofasteril and SkinNeutrAll) exhibited this ski slope effect, underscoring DNA damage. While not impeding the recovery of complete profiles, the ski slope effect was also observed on the electropherograms of undiluted blood samples decontaminated with GD6.

For diluted blood and the saliva samples, the ski slope effect was again visible for Wofasteril, Vaprox, GD6, and SkinNeutrAll in alignment with DI values $\geq$4, leading to partial profile recovery, as anticipated using the Vernarecci approach [52].

RSDL exposed saliva samples showed additional signs of the ski slope effect. One extraction of undiluted and diluted saliva exposed to RSDL presented strong degradation resulting in a partial profile for the undiluted sample and no profile for the diluted one.

Vaprox exposed samples demonstrated a decrease in profile recovery with decreasing DNA quantity. While three partial profiles with reasonable amounts of loci were recovered from undiluted blood samples, no profiles could be obtained from diluted saliva samples. Alldecont and GD6 showed similar degradation trends to Vaprox. All three extractions yielded full profiles for undiluted blood samples for both decontaminants. However, for diluted saliva, Alldecont allowed only one complete profile recovery and two no profiles, while GD6 resulted in partial profiles for all three extractions. As the availability of genetic material decreased, the chances of profile recovery diminished for these three decontaminants. Moreover, the available DNA was more degraded, as shown by higher instances of allele drop-out in the longer amplicons for these decontaminants.

SkinNeutrAll and Wofasteril had on the other hand similar trends with reasonable high amount of DNA recovered for both blood and saliva but with high DI values associated. This resulted in partial profiles and visually decreasing allele size response for longer amplicons markers.

4. Discussion

In the context of CBRN (Chemical, Biological, Radiological, and Nuclear) scenarios, effective triage of evidence is critical to prioritize resources and streamline the analysis process. This is particularly important in high-priority situations with multiple challenges. Employing decontaminants plays a significant role in this process, aiming to remove contaminants (hazardous agents), rapidly and effectively to ascertain safety and security, sometimes not prioritizing forensic relevant items.

Several studies have investigated the impact of degraded or complex samples on the ability to perform subsequent DNA profiling [6,19, 22–24,68–74]. The trends observed through our experiments are consistent with existing research [25–28,33,35], emphasizing the efficacy of specific decontaminants in removing DNA. Particularly chlorine and peroxide containing decontaminants affected DNA profiling negatively. Virkon decontamination resulted in minimal DNA recovery, aligning with the findings of Nilsson et al.'s study [28], which found Virkon to be the most effective method for DNA removal.

A distinction between gaseous and liquid forms in oxidative reactions, particularly concerning hydrogen peroxide, was reported by Finnegan et al. [75]. Due to material limitations, we did not assess vaporous decontaminants. It is noteworthy that various vaporous decontamination methods within a Vacuum Decontamination Chamber were investigated in the GIFT project [76].

Physical removal methods, such as water, soap water, and isopropanol, generally did not hinder DNA profiling. However, one extraction post-soap exposure and one extraction post-isopropanol exposure did not yield any DNA. In real-case scenarios, it's important to consider that the physical cleaning action of agents often involves a flow which may introduce a mechanical removal aspect. This may potentially complicate DNA recovery, although in our study, the impact appeared to be minimal. The contact time of these decontaminants can also play a key role since water can induce hydrolysis reactions on DNA if there is prolonged contact time.

SkinNeutrAll is a decontaminant designed to act on various Toxic industrial chemicals (TIC) and could be particularly relevant in clandestine laboratory discovery of chemical accidents [77]. Our results showed a deleterious effect on subsequent STR typing with mostly partial profiles recovered. However, most of these partial profiles conveyed sufficient information to be useable for comparison purposes.

Although DI values of GD6 and RSDL suggested DNA degradation, overall good profiles (complete or partial profile with high marker number) could be recovered, and the degradation affected profiling increasingly with decreasing DNA quantity. Additionally, GDS2000 did not impact DNA profiling whatsoever, regardless of the quantity.

Similarly, the powders showed little effect on the possibilities of DNA profiling.

A limitation of the study can be the applied contact time with the decontaminants. The direct exposure of blood and saliva samples to the decontaminants was brief, in accordance with the manufacturers' specified efficiency time (Table 1). While first responders may orientate the application time of the decontaminants based on manufacturers' instruction, practice may impede different realities. Yet, subsequently to the decontamination application, the samples were in our case, allowed to dry for 24 hours, during which residual decontaminants could still exert their effects on the samples. Although this time frame is relatively short, it is a plausible time frame in the context of a CBRN event where DNA samples could be considered of high priority and analysed rapidly after collection.

Our study allowed to reassess and emphasis that quantification results alone are not a reliable predictor for complete profile recovery. In some instances, low DNA quantities were recovered but complete profiles obtained, and conversely, in other cases, high amount of DNA was recovered but only partial profile obtained (Fig. 4). Thus, it is essential to consider the interplay between quantity and degradation in order to assess the impact of particular situations, in our case decontamination procedures.

Combining quantitation and degradation index values acquired by the qPCR quantification kits can provide steady evaluation for real cases allowing for good triage in high volume sample situations. This information aids in guiding the selection of optimal criteria for STR analysis, thereby improving the likelihood of successful profiling. In cases of degraded or low quantity DNA, customizing targets through the specialized amplification of short STRs or employing alternative methods to nuclear DNA STR typing may be envisioned.

Furthermore, our findings suggest that the approach proposed by Vernarecci et al.'s [52], using a degradation index threshold value of 4 for the Quantifiler Trio quantification kit, is relevant. This approach allows for flexibility, accommodating samples that may naturally be more degraded, such as saliva.

When it comes to collecting forensic DNA specimens, the focus is frequently on touch DNA rather than blood or saliva. The quantities anticipated with touch DNA are notably lower than those recovered from saliva and blood, suggesting that the effect of decontaminants may be greater for this type of specimens. Moreover, DNA in blood and saliva is largely enveloped by native tissues, which may serve as a protective barrier against reactive species.

Nevertheless, tendencies can be drawn from this study: decontamination of biological agents using hydrogen peroxide, peracetic acid or peroxymonosulfate oxidation (e.g. Vaprox, Wofasteril, and Virkon) generally impedes subsequent DNA profiling, as does the use of chlorinated decontaminants (e.g., bleach, Alldecont, BX24). On the other hand, organic based chemical agent decontaminants (i.e. RSDL, GD6 and GDS2000) or chemical powder decontaminants (e.g. FastAct and CHpowder) are not likely to hamper DNA profiling and there are good chances of obtaining usable profiles.

It is important to note that our experiments were conducted without prior exposure of the samples to any chemical or biological agents.

Consequently, the results obtained do not account for the potential cumulative interaction of these decontaminants with DNA in the presence of the anticipated biological or chemical contaminants. The variety of these agents is extensive, making their interactions challenging to predict.

5. Conclusion

In the context of CBRN scenarios, effective forensic items triage is crucial, and decontamination plays a significant role. Our study has revealed the varying impacts that different decontamination procedures have on forensic DNA profiling, with hypochlorite and peroxide based decontaminants showing damaging effects. Notably, Virkon exhibited minimal DNA recovery. Quantitative and Degradation Index values proved valuable for guiding STR analysis criteria. Decontamination with hydrogen peroxide or peroxymonosulphate-based oxidants generally hindered DNA profiling, while organic-based decontaminants had a less pronounced impact. However, the study acknowledges certain limitations, including brief exposure time and challenges in predicting cumulative interactions of contaminants and decontaminants with DNA. Overall, the research highlights the complexity of balancing decontamination efficacy with the preservation of forensic DNA traces in CBRN scenarios.

Ethical approval and consent to participate

The study was approved by the institutional ethical review board of the faculty of law, criminal science, and public administration (CER-FDCA) of the University of Lausanne, Switzerland (Number: E_FDCA_052023_00001, issued on 05.06.2023) and informed consent was obtained from the donors.

CRediT authorship contribution statement

Olivier Delémont: Writing – review & editing, Supervision, Methodology, Conceptualization. **Christophe Curty:** Writing – review & editing, Resources, Methodology, Conceptualization. **Natalie Kummer:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Isabelle Radgen-Morvant:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT 3.5 in order to proofread parts of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2024.112206.

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