

Recovery of DNA from Fingerprints on Enhanced Different Paper Types

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Citation: Zaghloul NM, Samir T, Megahed HM (2019) Recovery of DNA from Fingerprints on Enhanced Different Paper Types. J Forensic Sci Criminol 7(2): 205

Abstract

Background: Documents are commonly met in threats, kidnapping, and extortion at crime scenes. Perpetrators may handle papers, so that it may contain incriminating evidence. DNA recovery methods from fingerprints on papers are a specific area of interest to law enforcement personnel. Recovery methods, such as swabbing of surfaces, are destructive to fingerprints, so visualization of fingerprint details must occur first to maximize evidence recovery. DNA is possible to recover from fingerprints after enhancement, but the process could interfere with subsequent DNA typing according to DNA quantity, quality, and time lapse after enhancement.

Objective: The study aimed to define which paper type is likely to yield higher DNA recovery from fingerprints following enhancement by standard fingerprint enhancing reagents (Ninhydrin and 1,8-diazafluorene-9-one =DFO). The effect of Ninhydrin and DFO on the recovered DNA was also evaluated.

Method: Various types of paper with deposited fingerprints were processed for DNA extraction and quantification, following standard fingerprint enhancing procedures (Ninhydrin or DFO).

Results: Plain untreated (control) paper showed the highest DNA yield compared to treated different paper types. Magazine paper showed the highest amount of DNA recovery followed by office paper and finally newspapers. Developing fingerprints with DFO or Ninhydrin significantly decreased the yield of DNA from various types of paper. However, the effect of enhancing reagents was different from one paper type to another.

Conclusion: Enhancing methods applied to paper substrates lowered the quantity but did not inhibit successive DNA extraction or quantification. Magazines allowed higher recovery of transferred DNA than office or newspapers. Fingerprints developed with DFO showed slightly higher DNA yield compared to those developed with Ninhydrin on office and newspaper, respectively. Standard latent fingerprint development techniques used on paper were found to have a significant impact on the amount of DNA recovered.

Keywords: DNA Recovery; Touched Documents; Fingerprint Enhancement Reagents; Ninhydrin; DFO

List of abbreviation: DFO: 1,8-diazafluorene-9-one; PBS: Phosphate Buffered Saline

Introduction

In some committed crimes, whether, against property (theft, robbery) or against the person (physical violence, sexual violence, murder), the perpetrators are not punished, since traces with analyzable biological material that could identify them cannot be found [1,2].

Most trace pieces of evidence found in crime scenes are human fluids (blood, saliva, semen) and impressions like fingerprints. Fingerprints are of the most important and abundant evidence available [3]. Touched documents as letters are frequently met in cases of kidnapping, extortion, and threats. Likewise, the paper is often found at crime scenes, which can be found in the form of newspapers, magazines, envelopes, A4 office paper or tissues, all of which may have been touched by the committer and therefore may contain convicting evidence [4].

Latent fingerprint on porous surfaces as forensic evidence are often used as tools for identification, but sometimes these procedures are not sufficient to give an excellent individualizing image. Therefore, fingerprints can be used for DNA identification, serving as a source of biological matrix for DNA typing utilizing the small genome quantity that remains in the pyknotic nucleus of the stratum corneum of the shed epithelial cells. Touch DNA refers to the DNA retrieved from epithelial skin cells sheds, left after when individual touches or comes in contact with items such as weapons, clothes, documents, papers or other objects [5,6].

In the judicial process, the dactyloscopy approaches usually precede DNA typing, and it is applicable to study the effects of fingerprint augmentation systems on subsequent DNA profiling since the quantity of DNA in latent fingermarks varies remarkably [3].

However, DNA recovery methods, such as swabbing of surfaces, are usually destructive to fingerprints and so visualization and recording of fingerprint must occur first to maximize evidence recovery [7]. DNA recovery after fingerprint enhancement techniques is approached with several considerations based on several variables which include the surface type, collecting method, the development method required, extraction method, time lapsed after deposition and which evidence type will be more valued. Various chemical enhancement techniques are available to facilitate the visibility of the fingerprint pattern, which depends on the surface type. Ninhydrin and DFO are effective treatment for use on all absorbent porous surfaces, including raw wood, cardboard, paper, and matt painted walls. It has been recognized that it is likely to have a DNA type from a fingerprint after treatment, but this process might affect the subsequent DNA profiling [3,8-12]. Several analyses have been directed to assess the effect of fingermark enhancing reagents on the quantity and quality of DNA recovered for subsequent DNA typing. However, research on the recovery of DNA from touched papers is rather limited.

The current study aimed to define which paper type is likely to yield higher DNA from fingerprints, following enhancement by standard fingerprint enhancing reagents: Ninhydrin and (DFO). Moreover, to evaluate the effect of ninhydrin and DFO on the recovered DNA.

Materials and Methods

Ethical Consideration

Ethical approval was taken from the Ethics committee of the Medicine College in Misr University for Science and Technology, and the ethical committee of Alexandria University. (IRB NO: 00007555, FWA NO: 00018699). The procedures and the research goals were explained to all the volunteers before beginning the study. The fingerprints were kept anonymous, and all the fingerprint samples were destroyed at the end of the study.

Study Design and Settings

Collection of Fingerprints: Volunteers were asked to wash their hands with water and liquid soaps (germ protection hand wash) to remove any foreign cellular debris. This procedure was applied to avoid interference of extra and inter-individual DNA secondary transfer. Fingerprints of four volunteers were deposited on pieces of paper 80mm x 30mm of each of the following paper types:

- 1 Standard office paper (Xerox 80gm/m2) commonly used paper type was selected,
- 2 Magazine paper,
- 3 Newspaper.

Demographic data of the four volunteers was as follows: four adult males of age group between 24-33 years with a mean of 27.75±4.85 years. Exclusion criteria included; any skin disease, injuries to fingers, burns, and aggressive manual workers and corrosive exposure. Each individual was asked to rub their fingers over their face for a short period, (friction process as a manner of contact significantly increased the rate of transfer, touching their face, eyes, and nose). They are more likely to pick up DNA from those areas and transfer to other objects through touch). Then they were asked to firmly press their fingers (index, middle, ring and little finger) of washed hand for 30 seconds on paper [6,13].

Following the same procedure, each volunteer deposited nine sets of fingerprints, three sets on each paper type. All the paper pieces used to collect fingerprints were irradiated for 30 min in UV-crosslinker cabinet to eliminate any DNA contamination before fingerprint deposition [14].

Fingerprint Enhancement: The total of 36 sets of fingerprints collected was divided into three groups, and each group consists of three sets of fingerprints (one set on each paper type) for the four volunteers. The groups were designed as follows:

Fingerprint Group I: Left untreated as the control for all three paper types. (4 for the three paper type: 4x3 = 12 samples.

Fingerprint Group II: It was treated with DFO spray-Ready to use (code 28436, DH scientific[®]) as described in the Home Office Manual for Fingerprint Development (12 samples) [15].

Fingerprint Group III: It was treated with ninhydrin. (12 samples: 4x3). Also, as described in the Manual for Fingerprint Development. (12X3 = 36) [15].

(Ninhydrin crystals (151173-10G Sigma-Aldrich^{*}) was dissolved in 20 ml methanol 99% (Sigma-Aldrich^{*}) with minimum stirring as required to get complete dissolution, then made up to 100 ml with methanol and stored in a dark container).

Extraction method evaluation: The three groups of fingerprints were processed for DNA extraction using the QIA amp® DNA mini kit (QIAGEN*). The extraction method was performed by cutting the fingerprints from test papers in small fragments and incubates it in lysis buffer before proceeding with ethanol precipitation. In brief, the fingerprint areas were cut into small fragments approximately 5mm x 5mm portions, and inserted in 2 ml microcentrifuge tubes and PBS was added. The fragments were incubated in extraction buffer at 56 °C for 15 min. After incubation, tubes were centrifuged to collect any drops on walls or cap and the solution was transferred to a fresh tube. Ethanol was then added and extraction was immediately proceeded according to the manufacturer instructions manual. The DNA from each sample was finally eluted in 15 uL milliQ water. The samples were quantified using the Quantifiler* Human Quantification kit (Applied Biosystems*) real-time PCR method, as per the manufacturer's guidelines [16-19].

Methods of Statistical Analysis

The data were collected and stored into a personal computer. Statistical analysis was done using Statistical Package for Social Sciences (SPSS/version 21) software. Data were expressed as mean \pm standard deviation (SD), for more than two groups ANOVA test was used, followed by Post Hoc test to compare between each two groups. A p value less than 0.05 was considered statistically significant. The confidence intervals at 95.0% in our study were 3.

Sample size calculation was conducted using Epi-save software to perform a comparative study and detect the difference in DNA recovery from fingerprints on treated different paper types.

Sample size was estimated to be 36 set of fingerprints, and that was done in the study. The estimated sample size is made at assumption of 95% confidence level and 80% power of study.

Results

Effect of Paper Type on the DNA Recovery

The data of DNA recovery from each type of paper using different treatments for fingerprint recovery is shown in Table 1. Regarding the type of treatment material used, DFO spray, and Ninhydrin were used for fingerprint enhancement, and no material was used in undeveloped fingerprints in the control group.

Treatment Types Of paper	Control (without treatment)	DFO	Ninhydrin	ANOVA 1 p	P1 P2 P3
Magazine paper	0.69±0.042	0.262±0.031	0.256±0.0112	12.32 0.0003*	0.001* 0.001* 0.645
Office paper	0.276±0.009	0.072±0.007	0.068±0.009	7.13 0.009*	0.001* 0.0036 0.241
News paper	0.136±0.008	0.015±0.0066	0.011±0.054	6.25 0.013*	0.0036* 0.013* 0.611
ANOVA 2 p	10.65 0.003*	6.71 0.011*	7.65 0.006*		
P4 P5 P6	0.002* 0.001* 0.031	0.001* 0.001* 0.015*	0.002* 0.001* 0.035*		

ANOVA 1: To compare between different materials used

ANOVA 2: To compare between the different papers used in the same method of extraction

*: Statistically significant at $p \le 0.05$ LSD test was done by using Post Hoc Test to test the difference between each two groups.

P1 comparison between control and DFO P2 comparison between control and Ninhydrin

P3 comparison between DFO and ninhydrin

P4 comparison between magazine paper and office paper

P5 comparison between magazine paper and news paper

P6 comparison between office paper and news paper

Table 1: Comparison between different studied groups regarding the quantity of DNA recov-

ery (ng/µl) from various types of paper after extraction

Regarding the amount of DNA recovered from each paper type is observed in Figure 1 and Table 1. In Group I (untreated control group), magazine papers showed the highest amount of recovered DNA (0.69 ± 0.042 ng/ul), followed by office paper (0.276 ± 0.009 ng/ul) and finally, newspapers showed the least amount of DNA recovered (0.136±0.008 ng/ul).

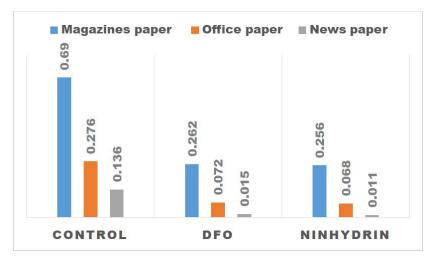


Figure 1: Comparison between different studied groups regarding DNA recovery (ng/µl) from various types of paper

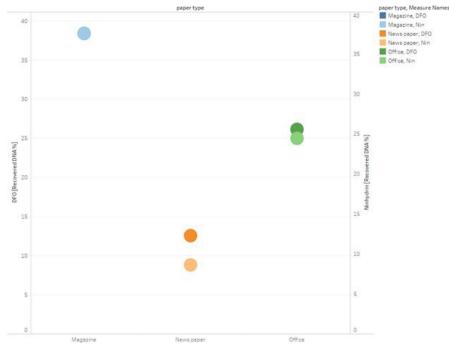
Effect of Fingerprint Enhancing on DNA Recovery

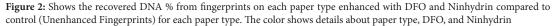
Magazine Paper: The DNA recovery in the control group was higher than in DFO and Ninhydrin group. On comparing the three types of fingerprint treatment, it was found that there was a highly significant increase in DNA recovery in magazine paper without treatment more than the DFO and Ninhydrin groups.

Office Paper (80gms): Also there was a highly significant increase in DNA recovery in the control group more than the DFO and Ninhydrin groups. There was no significant difference between DFO and Ninhydrin groups (p>0.05).

Newspaper: On comparing the three types of treatment on newspaper, it was found that there was a highly significant increase in DNA recovery in the control group compared to both DFO and Ninhydrin group.

Developing fingerprints with DFO or Ninhydrin significantly decreased the yield of DNA from various types of paper. However, the effect of enhancing reagents was different from one paper type to another (Figure 2). The percentage of DNA yields from different paper types enhanced with DFO or Ninhydrin compared to undeveloped prints (control) are shown in Table 2. In the case of Magazine paper, both DFO and Ninhydrin similarly caused more than 60% reduction in DNA yield compared to undeveloped fingerprints on the same paper type. However, in the case of newspapers, DFO treated fingerprints showed significantly higher DNA recovery than Ninhydrin (12.5% and 8.6%) from treated prints, respectively. In cases of office paper, fingerprints developed with DFO showed slightly higher DNA yield compared to those developed with Ninhydrin (26% and 24.4%) respectively.





Paper type	Fingerprint Enhancement reagent		
	DFO	Ninhydrin	
Magazine	38.34849%	37.49472%	
Office paper	26.096%	24.43366%	
Newspaper	12.55185%	8.601235%	
ivewspaper	12.3310370	0.00123370	

 Table 2: Shows DNA recovery percentage from different paper types with different enhancement reagents compared to control (un-developed fingerprints)

Discussion

Fingerprints have been considered as one of the most trustworthy biometric features for individual identification. Their efficacy has been further demonstrated by the new studies which have fruitfully shown the probability to attain surplus information about the giver [20-25].

With evolutions in modern DNA profiling technologies, it has been possible to have genetic information from samples with very minute DNA quantities. It has been shown that DNA can be obtained and profiled from objects which have been handled (such as a murder weapon, clothing victim, wallets, condoms, jewelry, lip cosmetics, glass, questioned documents, and papers. As fingerprint identification is important as DNA recovery is, so it was important to evaluate the effect and compatibility of chemical enhancement techniques of latent fingerprints (as Ninhydrin and DFO) with the DNA quantity recovered for subsequent DNA profiling. However, research on the recovery of DNA from touched documents after enhancement is rather inadequate [26-31].

So this study aimed to assess the effect of enhancement techniques on DNA recovered from fingerprints deposited on three different paper types.

The results of this research showed that DNA was successfully extracted using QIA amp[®] DNA mini kit. Magazine papers showed the highest amount of recovered DNA yielding approximately three to five folds compared to office papers and Newspaper respectively, followed by office paper and finally, newspapers showed the least amount of DNA recovered. On comparing the three types of fingerprint groups, it was found that there was a highly significant increase in DNA recovery in the untreated control groups (group I) for all types of paper more than the DFO and Ninhydrin groups (group II and III). Developing fingerprints with DFO or Ninhydrin significantly decreased the yield of DNA from various types of paper. However, the effect of enhancing reagents was different from one paper type to another.

Analyzing the results and comparing it with other studies, we found that Regarding the collection method of fingerprints for DNA analysis, in the existing study cutting method was used after chemical enhancement. Choosing this method come in accordance with the results of a thesis done at the University of Wolver Hampton in 2015, and showed that higher DNA recovery was obtained in samples collected using the cutting methods. However, the drawback of this method was mainly that the original evidence would be lost as a result of collection and analysis. As well as the limitation of the amount of the substrate that could be put into the extraction vessels. This variation of DNA might also be attributed to that tape lifting regains cells contained DNA from the surface-bound traces of the paper, leaving the deeper imprinted cells with its DNA contained materials. These deeper cells might be of sufficient evidential value [32].

The process of DNA recovery follows with DNA extraction from the substrate. The technique used in the extraction determines the efficiency of the process. In this study, QIA amp^{*} DNA mini kit (QIAGEN^{*}) was used successfully for DNA extraction from different paper substrates. Many researchers used other extraction methods such as 5% Chelex which outperformed organic methods in recovering DNA sample but from heels and toes [5,33]. In another study, the DN easy^{*} plant mini kit (QIAGEN^{*} was also used for DNA extraction from paper substrates and compared with the QIA amp^{*} mini kit used in the current study. and it was found to enhance DNA recovery from paper by over 150%. However, it has been suggested that the QIA shredder column included in the DNeasy^{*} plant mini kit, may have a strong aptitude in extracting DNA deposited on paper [10,11]. The kit column has a greater attraction for cellulose, and the lysis buffer solution that is supplied with the kit is thought to contain cellulase, making it highly discriminatory tool for use on plant material, such as paper [14,17,18].

Moreover, the nature of the substrate from which the DNA has been recovered could influence DNA extraction [34]. In Daly *et al.* [35] study, touch DNA was extracted from different substrates as hands to glass, fabric, and wood. While Ip *et al.* [36] studied the performance of five extraction methods on serially diluted blood and 76 simulated touch DNA sample. They found that QIA amp[®] DNA Investigator Kit, QIA symphony[®] DNA Investigator[®] Kit, and DNA IQTM yielded extracts with a higher success rate for the subsequent DNA typing analysis, as compared to Chelex[®]100 and QIA amp[®] DNA Blood Mini Kit.

One of the most significant factors of touch DNA is the type of surface on which DNA is deposited [28]. According to Wickenheiser [37], the smooth, plain and nonporous surfaces such as plastic and glass has less ability to retain DNA than a rough, absorbent and porous surface for example wood. This can be credited to the harsh scratch nature of the rough surface, which is likely to dislodge cells and therefore increasing chances of DNA retention. Wickenheiser [37], also argued that although it is true that more DNA was likely to be trapped on the coarse surface, the quantity of DNA that can be retrieved from such surfaces was lower and that might

be due to inefficient recovery processes from the rough, porous surface. However, Goray *et al.* [30] showed that the amount of DNA that can be retrieved from a cotton substrate (rough surface) on average was significantly higher as compared to the amount of DNA extracted from plastic (smooth surface). This implies that there is a higher preferential DNA deposition on the rough surface. This means that DNA persistence is higher on porous primary substrates, which are likely to surrender the deposited DNA more efficiently than non-porous surfaces.

Opposing this, Pesaresi *et al.* [38], suggested that smooth and nonporous surfaces such as glasses have better chances of holding more DNA than harsh porous surfaces such as untreated wood. This was attributed to the fact that smooth and nonporous surfaces increase the rate of perspirations during the contact, and therefore increasing the quantity of DNA left. Applying this principle may explain the current study results in which glossy magazine paper has higher DNA yield than office or newspaper. Magazine paper is coated by a smooth mixture of materials or polymer (chalk or china clay) to convey positive potentials to the paper, including weight, surface gloss, smoothness or decreased ink absorbency. The coating formulation may also contain chemical additives to give water resistance and wet strength to the paper. Maybe this was the cause that it has more ability to retain DNA than other types [39].

Concerning criminal case investigations, there are studies in many publications about the DNA being recovered from handled items, but few were done on different paper types. Papers are porous substrates of plant origin [40,41].

The present study demonstrated that different paper types showed variable DNA yield. This reflects the effect of the nature of the substrate on the recovery of DNA from different touched documents. Magazine paper showed the highest amount of recovered DNA, yielding approximately three to five folds compared to office papers and Newspaper respectively. This was in agreement with Sewell *et al.* [14]. Also, this study showed that treatment of samples with DFO or Ninhydrin for fingerprint enhancement significantly reduce the amount of DNA recovered. Nevertheless, enhancement reagents affected the DNA yield differently on different paper types. The DFO and Ninhydrin had a similar effect on the yield of DNA recovered from magazine paper. In case of Office paper developed with DFO showed a slightly higher amount of DNA compared to Ninhydrin development. The hypothesis that specific paper substrates interfere with the extraction of DNA was supported by these findings [14].

In contrast to Sewell *et al.* [14] study, the present study suggests that using DFO is more efficient to develop prints on Newspaper than Ninhydrin as it has a lower impact on DNA. Moreover, in cases of office paper, DFO is preferred as it showed a slightly higher yield compared to Ninhydrin developed prints.

In cases of fingerprints on Magazine paper, this study agreed with Sewell *et al.* [14] study that no significant differences were noted between Ninhydrin and DFO treatments in DNA yields, suggesting that choice of either reagent does not have a significant impact on DNA recovery from magazine paper.

The results obtained were partially in accordance with those of swell *et al.* [14] in which ninhydrin and DFO treatment were found to cause a 60% reduction in DNA recovery. Similarly, Schulz *et al.* [8] found that while DNA recovery may be lower after fingerprints enhancement, profiles can still be achieved, but in the present study, profiling was not done.

So the results of this study emphasize the utmost importance to have a fingermark enhancement protocol in place that not only augments the chances of reaching the fingermark match but also represents the finest method for DNA preservation. The protocol used in this study showed potential for obtaining DNA yields from treated different paper types. DFO showed a slightly higher amount of DNA recovery compared to Ninhydrin development, this finding have implications in choosing the technique of enhancement according to type of paper used criminal laboratories.

Conclusion

DNA was successfully extracted from different types of paper (magazine, office paper, and newspapers) after undergoing fingerprint enhancement techniques. When collecting DNA from paper touched pieces of evidence, the use of different methods such as chemical enhancement reagents (DFO and Ninhydrin) could have a direct impact on the quantity of DNA recovered. The type of the enhancing reagent may affect the yield. DFO showed a slightly higher amount of DNA recovery compared to Ninhydrin development for office and newspaper.

Recommendations

Based on the findings of the present study, it is recommended to expand the study on a large scale of samples and different substrates. Also, according to the results, laboratories and crime scene teams should reevaluate the methods and materials used to collect touched samples so that they are able to produce the best possible profiling results. Moreover, further work needed to be done to evaluate different factors that affect touch DNA, and choose the most suitable collection and extraction methods for different kind of surfaces, and different enhancement techniques of fingerprints.

Limitations

The current study was based on small sample size, and therefore, further work is needed that includes a larger sample size.

Conflict of Interest

The authors received no financial support for the research, authorship, or publication of this article.

Acknowledgment

The authors would like to thank the volunteers that donated samples for this work. Authors would also like to acknowledge the use of equipment provided by the College of Pharmacy at Misr University for science and technology.

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