

Analysis of DNA from Fired Cartridge Casings

S. Mawlood, L. Dennany, N. Watson, B. Pickard

Abstract—DNA analysis has been widely accepted as providing valuable evidence concerning the identity of the source of biological traces. Our work has showed that DNA samples can survive on cartridges even after firing. The study also raised the possibility of determining other information such as the age of the donor. Such information may be invaluable in certain cases where spent cartridges from automatic weapons are left behind at the scene of a crime. In spite of the nature of touch evidence and exposure to high chamber temperatures during shooting, we were still capable to retrieve enough DNA for profile typing. In order to estimate age of contributor, DNA methylation levels were analyzed using EpiTest system for retrieved DNA. However, results were not conclusive, due to low amount of input DNA.

Keywords—Age prediction, Fired cartridge, Trace DNA sample.

I. INTRODUCTION

THE primary objective of this investigation is to conduct an investigation on the survival of DNA in skin cell and sebaceous secretions when subjected to the stresses encountered in materials deposited on firearm cartridges when the cartridge has been fired. This information may assist the interpretation of results from analysing sebaceous secretions, i.e. touch, which are less readily controlled. It is necessary to determine first if the DNA sample that used in the tests will survive or not. Then to see if, with this survived evidence, we can build up a prediction of the contributor's physical feature, more precisely the age of contributor. As research shows that a code written into the body's epigenome the chemical tags that modifies DNA (methylation) can accurately tell the age of human tissues and cells [1], [2].

Therefore it aids the forensic scientists for a slightly more rapid pace during investigations. DNA analysis has been widely accepted as providing valuable evidence concerning the identity of the source of biological traces [3]. Recent work has raised the possibility of estimate some physical feature information such the age of the donor [4], [5].

It may also be possible to infer the ethnic origin of the donor [6]. Such information may be invaluable in certain cases where spent cartridges are left behind at the scene of a crime.

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Therefore, just a drop of DNA can gives a rough image of the age of an offender.

A crime investigator and/or forensic analyst may have numerous questions to answer during an investigation one of these might be, if DNA and epigenetically analyzable material can be obtained from fired cartridges. Professional investigators believe that there are no perfect crimes and according to Locard's exchange principle there will be some exhibits in crime scenes from perpetrator during handling any object present in the place [7]. So depending on both above facts, any possible touch DNA transferred to a cartridge case or bullet either before or during the gun loading process would be obliterated when the gun was discharged or handled.

Collection and extraction of DNA, from fired cases will simulate the finding of such items in the areas of a firearm crime scene and subsequently if possible, analysing the DNA methylation state from the DNA.

The challenging point in this study will be to assess if the temperature in the firearm chambers, pressure and potentially corrosive gasses generated during firing, will destroy or damage the DNA or can methylated DNA withstand this intense heat and survive.

Presently forensic scientists are hardly able to obtain genetic profiles from cells shed onto touched or handled objects by using advanced DNA typing methods as mentioned in the literature review [8]. This type of DNA is called "contact DNA or touches DNA" and currently investigators and attorneys send request to crime laboratory to try to find any evidence at a crime scene and analyze it [9].

Some common types of evidence at gun crime scenes are cartridge and firearms and are potential sources of contact DNA and perpetrators may not be wearing gloves all the time. Thus they may handle cartridges directly during loading of magazine. For this reason, in such type of firearm crime, forensic experts are often asked to swab cartridge cases for DNA evidence.

II. MATERIALS AND METHODS

A. Workflow Design

We have designed a study to evaluate the suitability of DNA derived from cartridge cases for use in criminal investigations and also to determine if epigenetic information can be analysed to get an idea about perpetrator's age. The workflow is showed as a diagram in Fig. 1.

B. Experimental Design

The experiment consisted of exposing the substrates to UV light before the experiment to destroy pre-existing DNA. After touching and loading all cartridges in to magazines or to the guns directly (Gunshot) by known shedder (to avoid DNA

shedder discrimination), under controlled circumstances, then shooting the weapons and testing spent cartridges to recover DNA and conduct the next generation matrix and analysis, these was done by using advance and automate technique for DNA recovery to find the best result and then applying the method finding the best result to real samples.

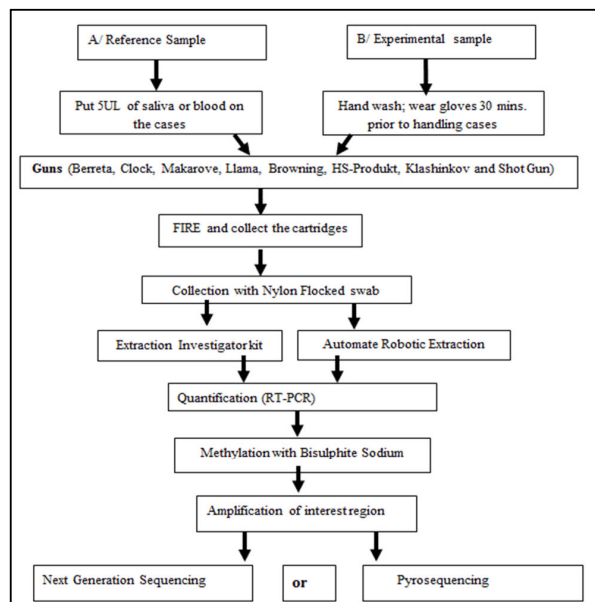


Fig. 1 Schematic diagram steps of the experiment: The diagram shows both reference and experimental sample steps from handling to analyze



Fig. 2 Eight different types of gun: The figure shows the weapon which were used in the study (from left to right). The top row is Berretta, Browning and Glock), in the middle row Gunshot one and double bore showed, whilst in the lower row, Llama, Kalashnikov and Makarov showed

A volunteer has been asked to load bullets into magazines (both cleaned before) of eight different types of weapons (shotguns has no magazine, hence the bullet was directly loaded in to the gun) the name and shape of the guns are summarized in Fig. 2. Then after firing process the fired cartridges were swabbed by Nylon Flocked Swab (forensiX) was chosen for the isolation step, because the swab has higher ability to retrieve and reserve biological fluids [10]. Then

extraction was done by using PrepFiler Express™ Forensic DNA Extraction Kit (Applied Biosystem). The advantages of this kit are its ability to cope with trace DNA and the extracted product will be free of PCR inhibitors. It was used with the AutoMate Express™ Forensic DNA Extraction System (Applied Biosystem) which minimizes the risk of contamination and transposition errors [11]. Magnetic filtration technology is used in this instrument, which traps magnetic particles against the sidewall of the pipetting tip. Furthermore there is no need for filtration and centrifugation steps during DNA extraction process, as simple bind-wash-elute procedure eliminates them. The tube rack and the tip are designed for stable mounting of elution tubes. To reduce the risk of contamination, whole liquid handling operations are performed away from the elution tubes and sample.

Automating the DNA isolation process on this system enables appropriate retrieval of DNA in high yields from the cartridge samples. In addition it removes most PCR inhibitors effectively[12].

C. Automate Express™ Forensic DNA Extraction System

To study fired cartridge case, AutoMate Express™ DNA Extraction system was chosen, which is an automated extraction platform that reduces DNA extraction times from many hours to 40 minutes. Extraction with robotic system allows researchers to check the reliability, reproducibility, sensitivity and limitations of the methods. This system is able to remove PCR inhibitors (dyes) from samples, and prevent sample-to-sample contamination [11], [12].

The most critical step of the analysis is the extraction of the DNA from forensic evidence sample, especially poor DNA samples [13]. The cells of interest (nucleated cells) contain other substances besides DNA such as, proteins, carbohydrates, lipids, etc. Therefore, the DNA molecules must be separated from this cellular material before it can be further examined [14]. Although, some other non-automated methods like organic extraction are used for DNA extraction. But multiple steps are required with these manual extractions can lead to contamination and/or loss of sample. It is also time consuming and does not remove all PCR inhibitors [13], [14].

Automated systems for DNA extraction have proven to be extremely useful, especially when processing reference samples (samples of known origin that contain ample amounts of DNA). Automated extraction is much faster - approximately thirty minutes for completion compared to other extraction methods. With automated systems there are less manipulations of the sample and no organic solvents are used. In the context of the laboratory, the absence of organic solvents makes the process safer for the person performing the extraction and the automation allows for faster extraction. Automated extraction methods use solid-phase extraction, compared to liquid-phase extraction, which is used in the organic extraction. Liquid-phase extraction requires multiple manipulations, requires organic solvents and is harder to automate, whereas solid-phase extraction is much easier to automate and does not require organic solvents.

The Applied Biosystem Corporation developed an automated DNA extraction system (Fig. 3). This automated system can purify high quality DNA from up to 13 samples simultaneously and in approximately thirty minutes. The system extracts DNA by magnetic bead particle technology.

D. QIAamp® DNA Investigator Kit

To isolate total DNA from buccal swab QIAamp® DNA Investigator Kit, lot #56504 (QIAGEN, Crawley, UK), was performed, following the manufacturer's guidelines.



Fig. 3 AutoMate Express™ DNA Extraction System: The figure show front side of the Robot Extraction System which used for DNA extraction from fired cases

E. DNA Amplification and Profiling

Some samples from each set were amplified using a 2720 thermal cycler (Life Technologies, UK) with the Investigator Human Identification PCR Kit (QIAGEN). While fired cartridge samples were processed for STR profiling using the Mini filer kit (Applied Biosystem, part #: 4374618) following the protocols as recommended by manufacture [15]. After running samples on the 3130 Genetic Analyzer (Applied Biosystem), the expected DNA profile was produced. There was no evidence of contamination.

F. Ammunition and Case Loading

Firstly a volunteer (known age and sex to the researcher) was asked to wear gloves for thirty minutes after hand washing. Then the volunteer was asked to handle ammunition directly and load ten bullets into a labelled and pre cleaned magazine of one of the weapons. To make the results of the experiment more accurate, the volunteer was asked to load ten bullets repeatedly and with each gun separately. A negative control was prepared by treating the cartridge with UV light using a cross-linker and a mock sample for positive control was prepared by handling a cleaned cartridge with a bare hand after firing.

G. Firing and Cartridge Collection

The surfaces of the firearms were cleaned prior to firing. Clean paper was laid on the floor to reduce contamination by extraneous DNA. A firearms examiner wearing gloves fired the weapons without touching the cartridges. Later on all the guns were prepared for firing and standard firearm safety protocol was followed as below:

The firing process was done at the firearm department in Erbil Forensic Laboratory which is suitable for this type of experiment. The instruction manual of the tested guns was well known and fully understood by the expert shooter before handling the weapons. Each of the weapons was thoroughly checked to make sure it was safe at all time before the test fire was carried out. The air gun was shot into a special standardized tank which is filled with water and never pointed at people. During handling of the air gun, fingers were not placed on the trigger unnecessarily unless when it was loaded and ready for firing. Hands were not permitted over the muzzle of the air gun. The air gun was placed on the proper table to prevent it from falling down and discharging accidentally. The air gun was never cocked until it was ready to be fired.

The ejected cartridge (discharged cartridge) cases were collected and placed into a labelled plastic bag until ready for swabbing. After collection, each shot shell case was swabbed with wet swab first with ultrapure water then followed by swabbing with a second sterile dry swab. After double swab technique the swabs were ready for extraction process.

H. Statistical Analysis

Microsoft Excel and Minitab® 16 software were used to conduct statistical analysis using one-way ANOVA test for significance between the results from different types of tested guns and the details is in Table II.

III. RESULTS AND DISCUSSION

A. Collected DNA

The first feature under investigation was the total yield amount of DNA obtained from cartridge's surface, to establish whether or not the DNA survive after firing through hot temperature and gas pressure. A volunteer was asked to load bullets into magazines of eight different guns. The weapons then shot and the fired cartridges were collected separately. In order to collect most biological genome on the case surfaces, ForensiX collection tube was used for swabbing as it has better ability to retrieve DNA comparing to normal swab (Swab study result). Automated instrument and PrepFiler Express BTA™ Forensic DNA extraction kit manufactured by Applied Biosystem (Life Technologies, Paisley, UK) was chosen for extraction step. This kit is designed for challenged forensic sample types like contact DNA. Therefore a powerful method to get the maximum amount and purity of DNA was used.

After DNA measurement with qPCR the result showed that cartridge of some weapons were more likely to produce viable amount of DNA than others as illustrated in (Fig. 4). The

results on shot shell surface of 12 Bore shotgun was around 0.9 ng (900 pg) of DNA even after shooting. While cartridges of Browning pistol represented the smallest amount of evidence items submitted for DNA analysis, around 0.1 ng (107 pg). When PCR amplification was done using Minifiler kit (Life Technologies, Paisley, UK), we were able to produce full DNA profile or at least partial profile for the majority shot shell of both types of shotgun.

We can explain the reasons that caused higher amounts of retrieved DNA from shot shell than other guns. First of all, the shot gun which we used was non-automatic weapon that means every single shot shell needed to be loaded before shooting and discharged again after firing. Hence there is no high gas pressure affect the evidence on surface of the ammunition. The size of the bullet is another reason which is bigger and longer (35mm) than other types of cartridge. Therefore there is more area surface between contacts during handling and this is Locard's exchange principle "Every contact leaves a trace" [7]. There is another possible explanation which is the fact that shotgun has no magazine. Thus the biological sample transfers directly to the gun. While in other automatic weapons which tested the, cartridges may lost a portion of biological fluid inside the magazine surface as in direct contact.

B. STR Analysis

The amount of collected DNA from cartridges of other tested guns was too low and not efficient to build a DNA profile. The 3010 GE analyzer result in most cases showed only partial profile. Most precisely in Beretta, Browning and

Kalashnikov weapons there was not clear peak in most if not all the loci using Mini Filer Kit as illustrated Fig. 5.

For STR profiling purpose, the Mini filer kit (Applied Biosystem) was used following the protocols as recommended by manufacture. This assay is optimized for genotyping degraded and/or inhibited DNA samples. In addition to sex determination (AMEL) locus, the kit amplifies eight autosomal STR loci. These loci (D13S17, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO and FGA) span a range between 70 to 283 nucleotides with the aid of non-nucleotide linkers to achieve appropriate spacing between loci. [15].

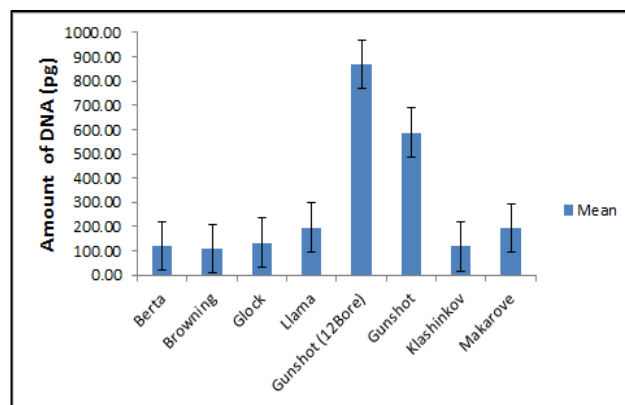


Fig. 4 Comparison data: The table compares the average (mean) amount of collected DNA (pg) from each of the eight tested guns

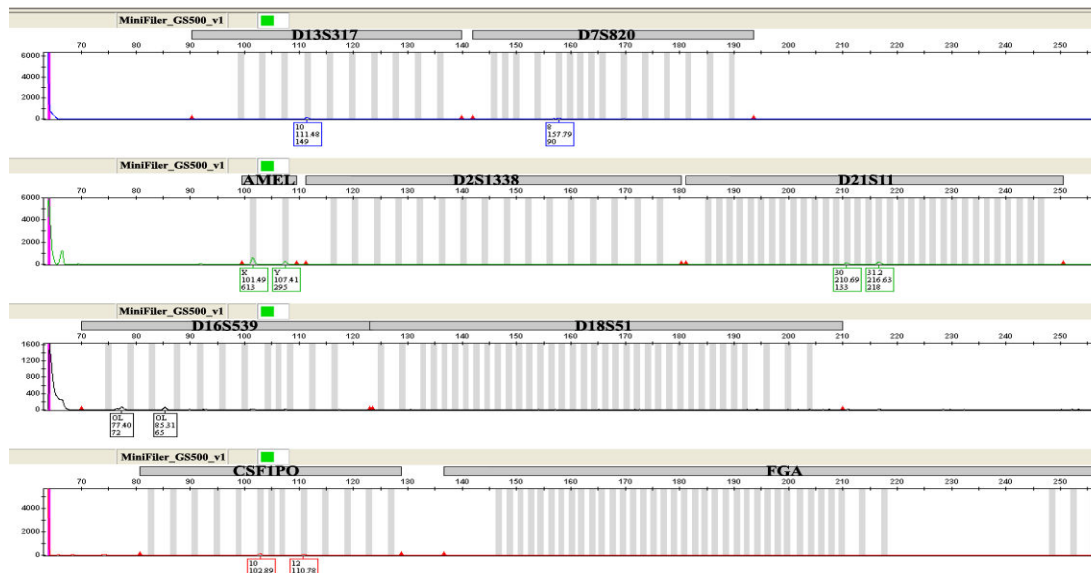


Fig. 5 MiniFilerTM profile obtained from a fired cartridge case: The figure shows the Genetic Electrophoresis result to build DNA profile from spent case of Kalashnikov, but only a few loci (D13S17, AMEL and CSF1PO) showed some poor peaks

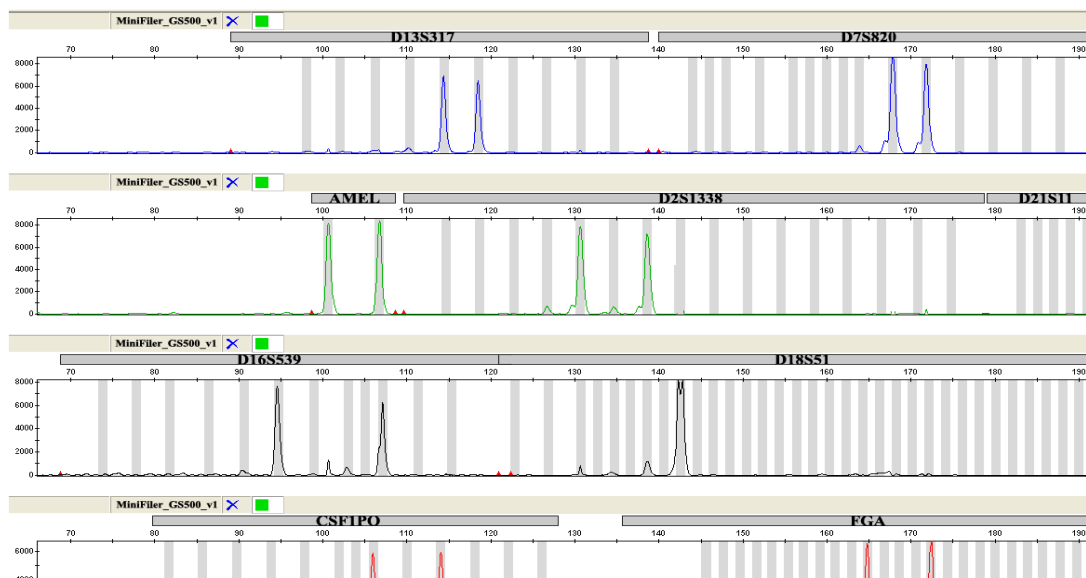


Fig. 6 MiniFilerTM profile obtained from a fired Shot shell: The figure shows a DNA profile obtained from fired shot shell of Shotgun-Bore. In addition to sex determination locus (Amelogenin), the sample yielded 7 STR (short tandem repeat) loci (except D21S11 locus)

The rifle's shot shells yielded enough DNA quantities that were suitable for further processing. Moreover, with GeneMapper® Software (Applied Biosystem) the STR result was more likely to produce viable DNA fingerprints and in three of ten cases nearly full DNA profile (except D21S11 locus which the span range is greater than 200 nt) were gained as shown in Fig. 6.

In all cases each weapon was cleaned well (out and inside) and exposed to UV light to avoid source of contamination of ammunition: i.e. contamination could be possible not only from the person who loaded the gun but also those people at manufacture who involved in making, packaging and handling the bullets before the experiment start.

The DNA profile result generally showed a single donor and if there was a complex mixture (e.g. more than two peaks in one locus) the experiment was repeated. Furthermore, even a shooter (known profile) could only be attributed to the mixture as a minor contributor of DNA still the result was not accepted.

Generally the DNA samples of the guns (except shotgun) did not yield sufficient DNA, or the profiles obtained were partial. Surprisingly among the eight tested weapons, Kalashnikov was found to yield DNA quantities less than 200 pg amplification threshold and the mean was only 58 pg as none of the spent cartridge cases produced sufficient amount of DNA (Appendix 1). One of the reason might be is that this weapon is considered as a selective firearm which has both semi-automatic and automatic mode thus beside the high temperature there is a high pressure gas during shooting and this could affect the DNA sample quality and quantity in negative way. Retrieved DNA samples from the cartridge cases that discharged from the all pistols that were examined in this study (Beretta, Browning, Glock, Liama and Makarov) showed nearly similar result with small portion difference due to the size and material composition of the cases, but the

retrieved DNA from any cartridges was not enough to build full eight STR loci. So, the results of this study support previous work which done by Polley et al. in firearm crime field. [16]. On the other hand, these findings of the current study are consistent with those of Rayan who found that DNA is not completely destroyed by the intense heat created during the firing process, although it has been estimated that internal temperatures of the firearm chambers can reach up to 1800 °C for between 0.5 and 5 ms [17].

It seems possible that the result of our study shows that bullets and cartridge casings (both fired and unfired) can routinely be examined in the DNA laboratory.

As there is a statistical difference among people in terms of DNA shedding (poor or good shedder), so might the volunteer in our experiment was not best shedder [18]. The cartridge case composition is another factor that can be taken in account which may due to variety amount of DNA as they made from different elements. Virtually all cartridge cases are made of brass (70% copper and 30% zinc). Some may also contain aluminum and few have a nickel coating [19]. So the surface or substrate type which contacted, the environmental factors and time contact have effect on the DNA yield. This is mean if the temperature was high like in summer then the loader hand will sweat and more cells are expected to transfer to the bullet surface [20]. Likewise as the time of contact increased there is more chance for cell exchange between two surface [7]. Moreover bullets within a box or lot unfortunately, do not have uniform composition, but there may be distinct groups of bullets within a box, so the fired cartridges were not the same all the time [21], Therefore different cartridge surface might have an effect on amount of yielded DNA.

C. Methylation Analysis of DNA Yielded from Cartridge

There is not a database for the whole population, so just with DNA profile we can only identify people who have

previous profile. Therefore, some information about external feature like ages of the criminals became pivotal. Although prediction of the age will not bring the person to justice directly, it could be aid the police in undertaking investigations at a slightly more rapid pace.

Using epigenetic signature differences to classify and distinguish age level is a current topic area of forensic interest. DNA methylation on cytosines is the best characterized among the epigenetic modifications which occurs through mammalian life time. The EpiTect Methyl II qPCR system (SA Bioscience, Qiagen, Hilden, Germany) is based on the quantitative detection of remaining input DNA within a sample population after treatment with methyl-sensitive and methyl-dependent enzymes [22], [23]. Primers are designed by an optimized computer algorithm to ensure that the amplicon contains cutting sites for both digestion types and are specifically designed for analysing the DNA methylation status of CpG islands. SYBR Green-based real time PCR detection is employed after digestion. To make the system work, each genomic DNA is subjected to four separate digestion treatments according to the manufacturer's protocol. The first condition is Mock digest (Mo) which means no enzymes were added and the product of this digestion represents the total amount of input DNA for real time PCR detection. The second condition is a methylation-sensitive digest (Ms) which cleaves unmethylated DNA and real time PCR therefore hypermethylated DNA. The methylation-dependent digest (Md) is the third condition, which digests methylated DNA and the qPCR detects the remaining unmethylated DNA. The final condition is for the double digest (Msd), both enzymes were added, and all DNA molecules (both methylated and unmethylated) are digested. This reaction measures the background fraction of input DNA refractory (R) to enzyme digestion and double digestion (Mdd). The relative amounts of DNA species from the targeted regions are assessed a comparative Δ CT calculation using an automated Excel-based data analysis template provided by the manufacturer.

Methylation analysis of collected DNA on ammunition was performed using EpiTect qPCR assay. Unfortunately, no successful results were obtained from this procedure. A possible explanation may be due to the lack of adequate input DNA as the manual recommend using 0.5-1 μ g, and not less than 150 ng of DNA, while in our study the maximum amount of retrieved DNA was from shot shell (1 ng) as shown in Table I.

A free Microsoft worksheet is available online to analysis the result of EpiTect system. There are some criteria requirements as a control for any result with this free software.

This Quality Control (QC) report calculates analytical window (W) and the percentage of DNA refractory (R) for each input DNA sample as illustrated in Fig. 7. If the analytical window is less than three ($W < 3$) meaning that the refractory DNA percentage is greater than 12.5 percent ($R > 12.5\%$), then the digestions are not complete and the analysis is reported as a "Failure" [24].

	A	B	C	D	E	F
1	Samples		SHOTGUN (12bore)		SHOTGUN	
2	Gene Symbol	Catalog Number	W	R	W	R
3	BCAS4-1	EPHS109350-1A	2.74	Failure	-4.45	Failure
4	KCNQ1DN	EPHS102112-1A	-4.81	Failure	-0.81	Failure
5	GRIA2	EPHS111325-1A	-0.93	Failure	2.71	Failure
6	TRIM58	EPHS101433-1A	1.6	Failure	1.4	Failure
7	SEC	EPHS115450-1A	11.78	0.028%	10.62	0.064%
8	DEC	EPHS115451-1A	5.58	2.091%	10.02	0.096%

Fig. 7 The QC data report worksheet: The figure shows the result of the quality control report and the value in analytical window (W) was abnormal (i.e. less than three) for the all age related genes. Similarly the refractory factor (R) of digestion was not complete for the input DNA means that the digestion efficiency was not high.

When the sample result passed QC report, then the template automatically calculates the methylation status of each gene of each sample as the percentage of methylated (M) and unmethylated (UM) DNA in the "Results" worksheet. The "Result" improve enzyme digestion's efficiency of the test. If the result was "Pass" for the control primer sets (SEC and DEC) which monitor both sensitive and dependent enzymes respectively, the result would be acceptable. That means real time PCR quantification and digestion steps were done successfully. In contrast the result worksheet for input DNA after digestion reported the result as failure in Fig. 8. Therefore the result was not acceptable due to the amount and quality of input DNA of the DNA sample.

Logically skin cells and saliva are the most probable source of DNA in a firearm cases and in a very few crime scenes blood can be find from fired casings, bullets and cartridges. But in our experiment the majority DNA source comes from skin cells and this is another reason why EpiTect qPCR system did not give result as the tested gene assays are optimized for age prediction in other types of tissues like blood and less so saliva, rather than skin cells.

	A	B	C	D	E	F
1	Samples		SHOTGUN (12bore)		SHOTGUN	
2	Gene Symbol	Catalog Number	UM	M	UM	M
3	BCAS4-1	EPHS109350-1A	Failure	Failure	Failure	Failure
4	KCNQ1DN	EPHS102112-1A	Failure	Failure	Failure	Failure
5	GRIA2	EPHS111325-1A	Failure	Failure	Failure	Failure
6	TRIM58	EPHS101433-1A	Failure	Failure	Failure	Failure
7	SEC	EPHS115450-1A	Pass		Pass	
8	DEC	EPHS115451-1A	Pass		Pass	

Fig. 8 Result worksheet: The figure illustrates the result worksheet of excel template which failed to show methylated (M) and unmethylated (UM) percentage of input DNA

Finally, there are numerous studies about DNA recovery from various locations of firearms, like ejected cartridge cases and non-spent cartridge or ammunition in general [16], [25], [26]. But still few laboratories report successful DNA typing with spent cartridge. These results of our study differ from some published report by [27], but they are consistent with those of [16], [25].

Future studies need to develop the EpiTect qPCR assay with more appropriate application method and investigating into ways of decreasing amount of input DNA. Furthermore a good epigenetic aging signature at a single gene that provides an accurate biomarker to predict the state of age needs to be

found, rather than the current multiple gene age signature process. This would remove the need to divide the amount of recovered DNA into six equal parts (four genes and two controls) as required in the assay.

APPENDIX

TABLE I

THE CARTRIDGE SIZE AND DETAILS OF DNA AMOUNT WHICH RETRIEVED FROM EACH FIRED CARTRIDGE OF EIGHT TESTED GUNS

Gun Type	Cartridge Size	Amount of DNA (picogram) retrieved from fired cartridge										Mean
Berta	9x19 mm	9.5	327.15	26.07	252.65	62.05	27.05	52.3	346.1	1.6	82	118.73
Browning	9x19 mm	135	14	24	0.7	8.5	3.1	240	195	50	405	107.53
Glock	9x19 mm	105.15	82.6	138.75	159.85	40.68	19.53	142.5	137.5	215.1	297	133.88
Kalashnikov	7.65 mm	17	49.6	6.5	180	93.5	26.5	29	6.5	25	146	118.11
Llama	7.65 mm	8	365	240	130	495	56	37	45.5	365	220	196.15
Makarov	9x18 mm	550	50	460	70	134	46.5	21.45	13.5	491.5	102	193.89
Shotgun	0.7 inch* 35mm	110	445	50	24	830	550	895	1000	395	450	587.66
Shotgun (Bore)	0.7 inch* 35mm	600	685	2045	1100	230	500	450	440	700	1955	870.5

TABLE II

ALL THE STATISTIC DETAILS OF THE EIGHT TESTED GUN INCLUDING AVERAGE AMOUNT OF RETRIEVED DNA (PG) FROM SPENT CASES AND SHOT SHELLS

Guns	N	Mean	SE Mean	StDev	Minimum	Maximum	Median
Berta	10	118.7	42.8	135.2	1.6	346.1	57.2
Browning	10	107.5	42.9	135.6	0.7	405.0	37.0
Glock	10	133.9	25.7	81.1	19.5	297.1	138.1
Llama	10	196.2	53.4	168.7	8.0	495.0	175.0
Gunshot-Bore	10	871	201	637	230	2000	643
Gunshot	10	574	119	375	24	1100	500
Kalashnikov	10	58.0	19.4	61.3	6.5	180	27.8
Makarov	10	193.9	68.2	215.6	13.5	550	86.0

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REFERENCES

- [1] Bocklandt S LW, Sehl ME, Sanchez FJ, Sinsheimer JS, Steve Horvath and Eric Vilain. Epigenetic Predictor of Age. PLoS ONE. 2011; 6(6).
- [2] Burgess DJ. Human epigenetics: Showing your age. Nature Reviews Genetics. 2012;14(1):6-.
- [3] Van Oorschot R, Ballantyne KN, Mitchell RJ. Forensic trace DNA: a review. Investig genet. 2010;1(1):14.
- [4] Meissner C, Ritz-Timme S. Molecular pathology and age estimation. Forensic science international. 2010;203(1-3):34-43.
- [5] Zubakov D, Liu F, van Zelm MC, Vermeulen J, Oostra BA, van Duijn CM, et al. Estimating human age from T-cell DNA rearrangements. Current Biology. 2010;20(22):R970-R1.
- [6] Evett I, Pinchin R, Buffery C. An investigation of the feasibility of inferring ethnic origin from DNA profiles. Journal of the Forensic Science Society. 1992;32(4):301-6.
- [7] Locard E. The analysis of dust traces. Part I. The American Journal of Police Science. 1930;1(3):276-98.
- [8] Wickenheiser RA. Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. Journal of forensic sciences. 2002;47(3):442.
- [9] Williamson AL. Touch DNA: Forensic Collection and Application to Investigations. J Assoc Crime Scene Reconstr. 2012;18(1).
- [10] Mawlood SK, Alrowaithi M, Watson N. Advantage of ForensiX Swabs in Retrieving and Preserving Biological Fluids. J Forensic Sci, May 2015, Vol. 60, No. 3. doi: 10.1111/1556-4029.12704.
- [11] Naseem M. DNA Extraction from Casework Samples using the Applied Biosystems® AutoMate Express™ Forensic DNA Extraction System 2011 (cited 214 10,15). Available from: <http://www.appliedbiosystems.com/ForensicNews-Past>
- [12] Liu JY, Zhong C, Holt A, Lagace R, Harrold M, Dixon AB, et al. AutoMate Express™ Forensic DNA Extraction System for the Extraction of Genomic DNA from Biological Samples*. Journal of forensic sciences. 2012;57(4):1022-30.
- [13] Montpetit SA, Fitch IT, O'Donnell PT. A simple automated instrument for DNA extraction in forensic casework. J Forensic Sci 2005. p. 555-63.
- [14] Butler JM, Shen Y, McCord BR. The development of reduced size STR amplicons as tools for analysis of degraded DNA. Journal of forensic sciences. 2003; 48(5):1054-64.
- [15] Biosystems A. AmpFLSTR® MiniFiler™ PCR Amplification Kit User Guide. July 2014.
- [16] Polley D, Mickiewicz P, Vaughn M, Miller T, Warburton R, Komonski D, et al. An investigation of DNA recovery from firearms and cartridge cases. Journal-Canadian Society of Forensic Science. 2006;39(4):217.
- [17] Ryan SR. Successful DNA profiling from fired casings, bullets and cartridges June 2012; vol.1, Issue 4. Available from: http://www.ryanforensicsdna.com/yahoo_site_admin/assets/docs/June_Newsletter.163101437.pdf
- [18] Dominick A, Welch L, Nic Daeid N, Bleay SM. Is there a relationship between fingerprint donation and DNA shedding? Journal of Forensic Identification. 2009;59(2):133.
- [19] Ravreby M. Analysis of long-range bullet entrance holes by atomic absorption spectrophotometry and scanning electron microscopy. Journal of forensic sciences. 1982;27(1):92-112.
- [20] Quinones I, Daniel B. Cell free DNA as a component of forensic evidence recovered from touched surfaces. Forensic Science International: Genetics. 2012;6(1):26-30.
- [21] Koons RD, Grant DM. Compositional variation in bullet lead manufacture. Journal of forensic sciences. 2002;47(5):950-8.
- [22] Sutherland E, Coe L, Raleigh EA. McrBC: a multisubunit GTP-dependent restriction endonuclease. Journal of molecular biology. 1992;225(2):327-48.

- [23] Stewart F, Raleigh E. Dependence of M_{er}BC cleavage on distance between recognition elements. *Biological chemistry*. 1997;379(4-5):611-6.
- [24] QIAGEN. EpiTect Methyl II PCR Assay Handbook. www.qiagen.com; 2012.
- [25] Horsman-Hall KM, Orihuela Y, Karczynski SL, Davis AL, Ban JD, Greenspoon SA. Development of STR profiles from firearms and fired cartridge cases. *Forensic Science International: Genetics*. 2009;3(4):242-50.
- [26] Nunn S. Touch DNA Collection Versus Firearm Fingerprinting: Comparing Evidence Production and Identification Outcomes. *Journal of forensic sciences*. 2013.
- [27] Branch L. Generating forensic DNA profiles from 'contact' DNA on cartridge cases and gun grips 2011.

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Within last decade experience in forensic analysis, he assessed every type of evidence from crime scenes (especially DNA profile) and interpretation of hundreds of cases in court has given him a deep understanding potential scientific methods to aid the investigation of 'cold cases'. This might include prediction of some physical feature of the perpetrator from basic (epi) genetic evidence: age prediction is one such direction receiving much research currently.

During current PhD research he has produced another publication in the *Journal of Forensic Science* and presented his works at several international conferences.