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Application of DNA methylation-based markers in identification of mixed body fluid evidences simulating crime scene scenarios

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Abstract

Background: Epigenetic modifications are heritable and follow a non-mendelian inheritance pattern. They do not alter the DNA sequence but affect the gene expression at the transcriptional level. DNA methylation is one of these epigenetic changes and it is characteristic to each tissue and shows specificity with respect to developmental stage and age. Due to its specificity and reliability, it has emerged as a valuable tool in forensic investigation. Biological samples, such as blood, saliva, semen, or hair found at the crime scene can be used to isolate DNA and study the methylation pattern. Recent developments in molecular biology techniques allowed the study of the effects of methylation in specific tissues. DNA methylation specificity is very intense. These specific markers can be used to identify the tissue type such as blood, saliva, or semen at the crime scene and helps in the identification of the culprit. The present study aimed to validate the use of DNA methylation body fluid-specific markers in the identification of peripheral blood, menstrual blood, and semen. Additionally, it aimed to investigate the potential use of such DNA methylation markers for the identification of different body fluids mixtures simulating forensic science scenarios. Different DNA methylation markers were studied in different body fluid samples (peripheral blood, menstrual blood, and semen) individually and as mixtures. DNA extraction and bisulfite conversion were performed and followed by real-time PCR.

Results: The results of real-time PCR and the statistical analysis showed that the SPERM2 marker was better than SEU2 in the identification of semen DNA in mixed samples. However, in the identification of individual semen samples, the later marker showed better results than the first one, whereas BLM1 and MENS1 markers were successful in identifying the peripheral and menstrual blood samples, respectively.

Conclusions: This data can be readily used and applied on different forensic samples for tissue identification. Further sequencing studies are strongly recommended.

Keywords: DNA, RNA, Body fluid identification, Epigenetics, DNA methylation, Bisulfite conversion, Forensic science, Sexual assault, DNA extraction, Real-time PCR, Mixed biological samples

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Background

Analysis of biological fluids recovered from the crime scene enables the investigating authorities to identify a suspect or victim or exonerate an innocent individual. Determining the type and origin of biological samples can be used to reconstruct the crime scene. It is not an easy task to identify and characterize a biological fluid due to its similarities with other biological fluids. When the identity of a fluid appears obvious, absolute confirmation about its nature is essential to be used as evidence in judicial courts. It is also important to establish whether the stain is composed of mixed fluids or the same fluid from different individuals (Gomes et al., 2011).

Several physical tests have been used to identify and characterize the nature of a particular fluid. The external examination may reveal the nature of the identity of the fluid, but this is not enough when dealing with a mixture of fluids or fluids from multiple donors (Gomes et al., 2011). So, careful analysis using advanced molecular techniques is necessary to establish the precise sequence of events. Two levels of testing are commonly performed to verify the identity of body fluids that include both presumptive tests and confirmatory tests. However, presumptive tests have limited specificity, so they just serve as initial screening tests which can narrow down the list of suspects to a particular group. Meanwhile, confirmatory tests are very specific and provide absolute identification of tissues.

Most of the screening tests are destructive in nature. So, the sample cannot be recovered for further analysis. So, it is considered a problem when only a limited amount of samples is available. Presumptive tests have been in use since several decades. Identification of heme in blood, amylase in saliva, and phosphatase in semen are few examples of presumptive tests. The major disadvantages of these presumptive tests are the destruction of the sample, the clinical investigator has to opt for a specific test if there is only a limited sample available, and most of them are based on the enzymatic or immunologic activities. These assays depend upon the stability of the target samples and require functional molecules in the sample which may not be a common occurrence in a crime scene (Virkler and Lednev, 2009). In the cases of environmental degradation or damaged samples, it is very difficult to employ such procedures. These tests also show less specificity. Cross reaction with other molecular species may interfere with the final conclusion. Furthermore, most of the commercially available screening test kits are not quantitative in nature. They can just detect the presence or absence of the tested molecular species but cannot quantify them. So, there is no statistical accuracy associated with these techniques.

Recently, several methods have been developed that included the use of RNA as an absolute identification tool (Hanson and Ballantyne, 2013). However, RNA is known to be very unstable, and thus, it requires recovery of good samples from the crime scene in adequate amounts. Genome-wide studies can be performed with RNA, but it is very cost ineffective and time-consuming. Therefore, after several trial and error attempts, scientists have opted for DNA for the analysis of body fluids. DNA is the ideal source for body fluid identification because it is stable and genome information of humans as well as other related animals is well known. Each individual carries a unique DNA sequence which is of great significance in concluding ample evidence that the given sample belongs to a particular suspect or victim. DNA analysis provides quantitative results and no additional processing is required (An et al., 2012).

DNA methylation and histone modifications are the major epigenetic mechanisms found in eukaryotes. Both mechanisms do not alter the base-pairing capacity of individual bases, however, they alter the effectiveness of transcription machinery to the designated locus and hence affect its expression. Histone binding affinities to DNA can be altered by several covalent modifications such as methylation, phosphorylation, ubiquitination, and acetylation (Shen and Waterland, 2007). Each modification has a specific effect on gene expression. For example, methylation is often repressive in nature and acetylation serves as an activation signal. It also depends on which modification is occurring at what residue of the histone protein. In contrast to histone modifications, DNA methylation always leads to repression of the gene expression (Gaydos et al., 2014). Even in monozygotic twins who contain almost identical DNA sequences, the difference in their behavior and physiology is attributed to changes in DNA methylation patterns. So, DNA methylation does not change the genotype of an organism but can show the phenotype of a loss of function allele. Such alleles are referred to as epialleles (Jones, 2014). Each tissue type acquires different methylation patterns during its differentiation. So, these different methylation patterns can provide the basis for an assay to identify body fluids (Kader et al., 2019).

Methylation occurs specifically at 5′-CpG-3′ dinucleotide sequence where sequences are known as CpG islands and they are normally located at the transcriptional start sites (Varriale and Bernardi, 2010). In mammals, it has been reported that 70–80% of CpG islands can be methylated. CpG sites occur at a lower frequency than expected in the human genome. Since it has been very well established that CpG islands are clustered at the transcription start sites of genes, this information is also utilized for the annotation of new genes in recently sequenced genomes of other organisms. CpG islands are

characterized by the high frequency of CpG dinucleotide (4–6%) as expected to the normal frequency of less than 1%. Methylation along with histone modifications are integral components of gene imprinting (Nazarenko, 2015). DNA methylation is characterized by having a dynamic nature and can respond to multiple internal and external signals (Stower, 2012).

In forensic investigations, it is common to test for DNA to acquire information about criminals and help with uncovering evidence on various dangerous felonies. Blood samples, saliva, and other elements are known to be the most common forensic evidences and DNA has a major role to play for identification purposes. Forensic scientists can uncover DNA through used items such as clothes, gloves, condoms, and basic used items like ropes and wires. The biological samples acquired for DNA testing are easier to collect nowadays and can extend to low-level DNA from simply collecting very small skin samples or touching surfaces used by the criminal or victim (Buckleton, 2009).

Scientists have established various protocols for the isolation and characterization of DNA from biological fluids. The major disadvantage with sequencing methods is that it can differentiate one person from the other but cannot differentiate monozygotic twins, but still cannot distinguish different body fluids from the same individual as all the cells in a human body contain almost the same DNA sequence. That fact leads the scientists to propose the use of epigenetic markers for the identification of the type of tissue of an unknown human biological sample as well as determining the age of this sample. Additionally, these epigenetic marks can differentiate between monozygotic twins. These modifications are stable and heritable. It is a reliable technique to reach an outcome of investigation (Vidaki A. and Kayser M., 2018). Not only the sequence context, but also several external factors such as diet, age, and other lifestyle choices determine the patterns of DNA methylation in an individual. These differences can also be exploited in forensic and medical studies to identify the victim or culprit.

Regarding techniques that can be used for studying the methylation pattern, Bisulfite sequencing is one of the basic techniques. Bisulfite treatment introduces specific changes such as amination, sulfonation, and deamination at the methylated cytosine residues. Unmethylated cytosine residues are unaffected by the bisulfite treatment. In this procedure, the isolated DNA from the crime scene is subjected to bisulfite treatment. This converts all the methylated cytosines into uracil. After bisulfite treatment, the DNA sample is sent for DNA sequencing which reveals the position and pattern of cytosine methylation (Cai, 2005). In sequencing reaction, unmethylated cytosine is read as adenine in the antisense strand and as thymidine in the sense strand. In

most of the bisulfite sequencing reactions, sodium bisulfite is used. Recent methods use ammonium bisulfite which has higher solubility compared to sodium bisulfite. High concentration of bisulfite in the reaction mixture ensures complete conversion of all the methylated cytosine into uracil.

Due to its immense importance in forensic investigation and medicine, several advanced techniques are developed in recent times. These include methylation-sensitive restriction enzyme profiling, allele-specific bisulfite sequencing, pyrosequencing, and mass array. Bisulfite methylation studies are not only useful in identifying the origin of a biological sample, but it is also used to predict and conclude several disease occurrence states, viral infections, and drug addictions.

Moreover, real-time PCR, methylation-specific oligonucleotide blockers, and methylation-specific probe can be used for amplification of methylation-specific markers. This method can detect upto 30–60 pg of methylated DNA. This technique is known as Heavy Methyl technology and is shown to be successfully employed in detecting methylation patterns in very low amounts of serum samples from cancer patients (Cottrell et al., 2004).

In addition, the methylation pattern can be analyzed using pyrosequencing following bisulfite conversion (Tost and Gut, 2007). This provides accurate quantification of methylation in sequence context since traditional Sanger sequencing provides only semi-qualitative methylation data that might lead to inaccurate conclusions. Furthermore, mass spectrometry can be used to study methylated cytosine in short sequences. It is a highly accurate and reliable technique that can be used for smaller known sequences and can be applied to confirm the methylation patterns already identified by other methods. Moreover, a whole genome bisulfite sequencing can be a high throughput tool for methylation pattern determination.

In addition to all the previous techniques for studying DNA methylation pattern, other methods are also available like *HpaII* tiny fragment Enrichment by Ligation-mediated PCR Assay *(HELP assay)* (Suzuki and Greally, 2010), *Chip-on-Chip assay*, and *Methylated DNA immunoprecipitatio*n (Jacinto, Ballestar and Esteller, 2008).

Previous studies have identified several tissue-specific DNA methylation markers that can be used in forensic investigations for identification purposes and can also be informative when dealing with mixed biological samples (Lee et al., 2015). Among these tissue-specic markers are C200rf117, ZC3H12D, and BCAS4 that have been reported to be hypermethylated in blood, semen, and saliva, respectively.

Several other studies also have established numerous markers that can differentiate between closely related

tissues. For example, Blut 1-f and Blut 2-f are methylated in menstrual blood samples. But, Blut 1-f is unmethylated and Blut 2-f is methylated in pure venous blood. These markers can distinguish the origin of body fluids even in the mixed samples (Forat et al., 2016).

Using pyrosequencing and Illumina Human Methylation 450 K bead array, Park et al. (2014) have extensively studied DNA methylation patterns in four types of body fluids (blood, saliva, semen, and vaginal secretions). They have reported a total 286 differential methylation sites, of which eight sites have been validated and have been proven to have a high forensic significance. These sites are cg06379435 and cg08792630 for blood, cg26107890 and cg20691722 for saliva, cg23521140 and cg17610929 for semen, and cg01774894 and cg14991487 for vaginal secretions.

This project aimed to:

- Validate the use of DNA methylation body fluid specific markers in the identification of peripheral blood, menstrual blood, and semen.
- Investigate the potential use of such DNA methylation markers for the identification of different body fluids mixtures, simulating forensic science scenarios.

Methods

This project was carried out in a Biotechnology Research lab. A written consent was taken from 25 participants. From whom, peripheral blood was collected from 10 volunteers, semen was given by 7 volunteers, and ten women took a self-swab of menstrual blood. All the samples were stored at − 20 °C. DNA was extracted from all samples using QIAamp DNA Investigator kit (Qiagen) according to the manufacurer's instructions. After verification of successful DNA extraction using gel electrophoresis. DNA samples were subjected to bisulfite conversion using sodium bisulfite treatment which converts unmethylated cytosines into uracil, while the methylated ones remain unchanged. For this step, EpiTect Bisulfite Kit (Qiagen) was used according to the protocol given by the manufacturer. Afterwards, the DNA methylated markers for individual body fluids were amplified using real-time PCR. For peripheral blood, the identification marker used was Cg03363565, located on chromosome 16, and for menstrual blood, the marker named Cg09696411 located on chromosome 12 was amplified. Regarding semen identification, two markers were used; Cg05656364 (methylated marker), located on chromosome 2, and Cg11768416 (non-methylated marker), located on chromosome 5. The details of primers used are summarized in Table 1. The reaction setup involved the use of 10 μl of iTaq™ universal SYBR® Green supermix, 2 μl of primers and 2 μl of DNA in a total volume of 20 μl reaction mix. Thermal cyclic conditions were followed as given by Forat et al. (2016) and Vidaki et al. (2016).

Biological samples were then mixed in different proportions to simulate different forensic scenarios. Female peripheral blood was mixed with semen and menstrual blood was mixed with semen in three different ratios: 1: 1, 7:3, and 3:7. Furthermore, all three different body fluids were mixed together in the form of a full swab of menstrual blood mixed with 50% peripheral blood and 50% semen. DNA was then extracted from all mixed samples using QIAamp DNA Investigator kit (Qiagen). Afterwards, the DYS14 gene on Y-chromosome was PCR amplified (using forward primer 5′ CATCCAGA GCGTCCCTGG 3′ and reverse primer 5′ TTCCCCTT TGTTCCCCAAA 3′) to investigate the presence of male DNA in all biological mixtures. Lastly, DNA mixtures were subjected to bisulfite conversion, followed by real-time PCR amplification of different body fluids specific DNA methylation markers. Analysis of variance (ANOVA) statistical test was performed to detect any significant differences between the two semen-specific DNA methylation markers for identification of semen in mixed body fluids (Tables 2 and 3). The statistical test was conducted using SPSS.

Results

Gel electrophoresis results have shown successful DNA extraction from all individual samples of different body

Table 1 Details of primers used for individual body fluid DNA methylation markers

Marker	Primer	Sequence (5′–3′)
Cg09696411 (menstrual blood)	MENS1-F	GATTAGGTTTAGGGAAGTTTTTAT
	MENS1-R	ACC CTC TAA AAC TTA TAC TCC C
Cg13763232 (peripheral blood)	BLM1-F	TAGTTGATATTGGTTTGGTA
	BlM1-R	CAAATAACTCAATTTCTCTAC
Cg05656364 (semen)	Sperm2-F	ACT AAA ATC TAA ACT AAA AAC TAC CC
	Sperm2-R	ATA GTT TTG AGT TGT TTT GGT AGG TG
Cg11768416 (semen)	SEU2-F	GGAGGTTGTTTTTTTTTTGGTTT
	SEU2-R	CTACCAACACCTTCCTCC

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Table 2 ANOVA of results of amplification of the four body fluid-specific DNA methylation markers in different body fluids

Summary

Groups	Count	Sum	Average	Variance		
Peripheral blood (BLM1)	10	257.34	25.734	2.845538		
Menstrual blood (MENS1)	10	256.53	25.653	5.154668		
Semen (SPERM2)	7	177.31	25.33	9.559033		
Semen (SEU2)	5	104.76	20.952	8.80742		

ANOVA

Source of variation	SS	df	MS	F	P value	F crit
Between groups	91.83036	3	30.61012	5.207519	0.005523	2.946685
Within groups	164.5857	28	5.878062			
Total	256.4161	31				

fluids as well as all biological mixtures. In addition, male DNA was successfully identified in all mixtures of body fluids even in samples where the percentage of male participation was 30% of the total samples as shown by successful PCR amplification of the DYS14 gene (Fig. 1)

Real-time PCR amplification of BLM1 marker in individual peripheral blood samples was successful for all 10 samples with Cq values ranging from 24.33 to 28.22. Similarly, real-time PCR has shown successful amplification of MENS1 marker in all menstrual blood samples with Cq values ranging between 20.56 and 28.79. For real-time PCR amplification of SPERM2 marker in individual semen samples, it showed amplification with Cq values between 22.52 and 31.12. However, the SEU2 marker was amplified in semen samples by real-time PCR with a lower Cq value range (18.43–26.07). A comparison between the mean of Cq values among the four different DNA methylation markers in individual body fluid samples was shown in Fig. 2. Regarding real-time PCR amplification of DNA methylation markers in mixtures of biological smaples, the SPERM2 marker has

Table 3 ANOVA of results of amplification of the two semen-specific DNA methylation markers in mixed body fluids

Summary

Groups	Count	Sum	Average	Variance
Mix (SPERM2)	7	177.99	25.42714	6.831324
Mix (SEU2)	7	183.49	26.21286	2.991424

ANOVA

Source of variation	SS	df	MS	F	P value	F crit
Between groups	2.160714	1	2.160714	0.439941	0.51969	4.747225
Within groups	58.93649	12	4.911374			
Total	61.0972	13				

shown positive results in all 7 mixtures with Cq values ranged from 22.43 in mixture 7 (contained all three body fluids) to 29.40 in mixture 2 (contained 30% semen) with a mean of 25.4 approximately. Similar results have been reported when amplifying the SEU2 marker in all mixtures but with a slightly higher mean of Cq values (26.2) compared to all other methylation markers either in individual samples or in mixture samples (Figs. 2 and 3). By comparing results of real-time PCR amplification of both individual body fluid samples and biological mixture samples, it was shown that there was a significant difference in SEU2 marker compared to SPERM2 marker as shown in Figs. 4 and 5.

Discussion

DNA methylation is an epigenetic marker that does not change the sequence but exerts its inhibitory effect on gene transcription by recruiting inhibitory proteins. Unlike other epigenetic modifications, DNA methylation of a locus always results in gene repression. Cellular machinery uses methylation as a sensitive and effective method to regulate the expression of key genes during various developmental stages. They are sensitive to the health of individuals, exposure to mutagens, and several psychological factors as well. Each tissue contains
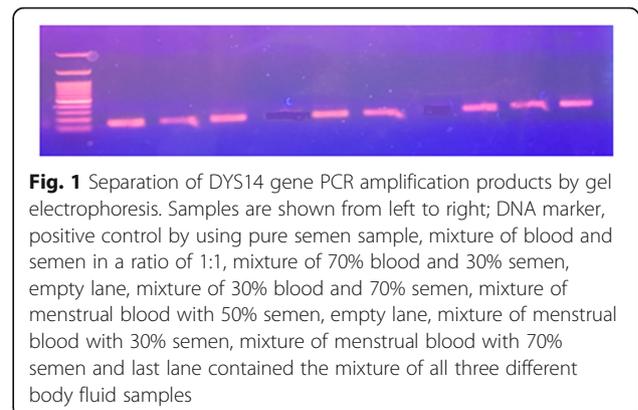

Fig. 1 Separation of DYS14 gene PCR amplification products by gel electrophoresis. Samples are shown from left to right; DNA marker, positive control by using pure semen sample, mixture of blood and semen in a ratio of 1:1, mixture of 70% blood and 30% semen, empty lane, mixture of 30% blood and 70% semen, mixture of menstrual blood with 50% semen, empty lane, mixture of menstrual blood with 30% semen, mixture of menstrual blood with 70% semen and last lane contained the mixture of all three different body fluid samples
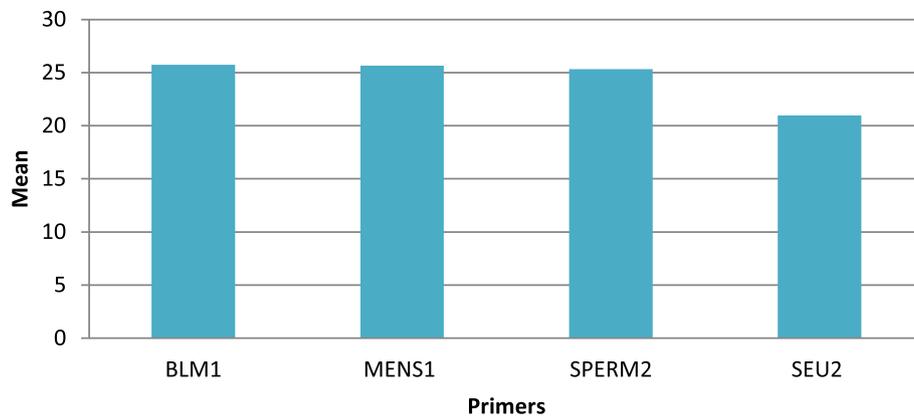
Fig. 2 Comparison between the mean of Cq values in individual body fluid samples

specific methylation signals during its development. So, using these specific markers, it is possible to identify the source of the sample. This data coupled with other experimental evidence can be used to solve criminal cases. Due to its growing importance, several methods have been developed to detect DNA methylation. The basic method is the treatment with bisulfite followed by sequencing. Several genome-wide analysis studies have established and characterized the methylation patterns of various tissues. So, these patterns can be used as a marker in forensic investigation to identify the body fluid and person (Stower, 2012)

DNA was extracted from all the individual and mixed samples successfully. DYS14 was successful in indicating the presence of semen DNA even if it was present in a small proportion.

The results showed that BLM1 is a good primer to be used for the detection of peripheral blood in the crime scene. Similarly, it was reported by Vidaki et al., 2016 that the BLM1 marker was highly detective of blood tissues while it was weak in detecting other tissues such as saliva and skin samples. The results confirmed that the MENS1 marker was successful in detecting the

menstrual blood samples. In the same way, it was reported by (Forat et al., 2016) that the MENS1 marker was exclusively methylated in menstrual blood and unmethylated in the other body fluids, and it was very sensitive to detect even a small amount of menstrual blood DNA.

The current study confirmed the successfulness of SPERM2 and SEU2 markers in detecting DNA in semen samples. Coinciding with these results, Forat et al. (2016) mentioned that SPERM2 can identify the presence of seminal fluids. Additionally, Vidaki et al., 2016 mentioned that SEU2 seemed to be highly specific markers for semen and the sensitivity of the proposed semen-specific methylation assays was assessed by analyzing decreasing amounts of starting DNA material (10 ng, 1 ng, 500 pg, 100 pg, 50 pg, and 10pg) in duplicate. successful amplification and the expected semen methylation pattern for SEU2 was obtained with as low as 50 pg of starting DNA.

Additionally, the study showed that SPERM2 and SUE2 primers could be used to indicate semen DNA in individual as well as mixed body fluid samples, even if it is present in minute amounts.
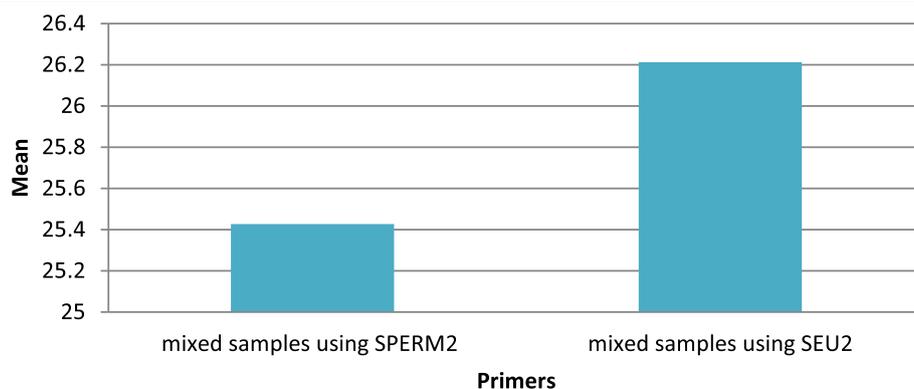

Fig. 3 Comparison between the mean of Cq values of SPERM2 and SEU2 methylation markers in biological mixtures
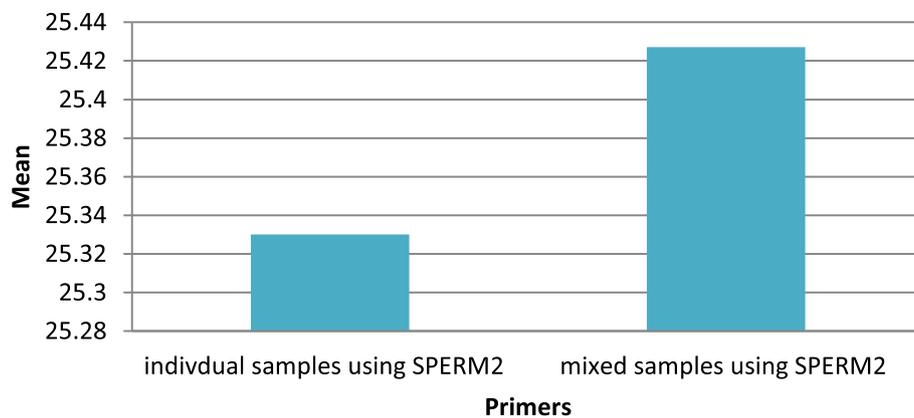
Fig. 4 Comparison between Cq values of SPERM2 amplification in individual semen samples and mixed body fluids

Based on the results obtained by this study, the best DNA methylation marker was SEU2 followed by BLM1 and MENS1. Analysis of variance has shown that the mean value was significantly different for the BLM1 marker that was used in peripheral blood samples compared to the MENS1 marker that was used in menstrual blood samples with a difference value of *$P < 2.3$. And when variance analysis was performed comparing SPER M2 and SEU2 markers, which were used to detect individual semen samples, a slight difference between values was detected (*$P < 0.75$). The results of that study showed that the SPERM2 marker was better than SEU2 when it is used in mixed samples to detect the male DNA. Analysis of variance was performed showing that the mean value was significantly different for SPERM2 compared to SEU2 with a difference value of *$P < \approx 4$.

By comparing the results of SPERM2 marker amplification in both individual samples as well as mixed samples, it was shown that individual samples had a lower mean with a value of 25.33 than mixed samples with a value of 25.427, which confirmed the effectiveness of using the SPERM2 marker in the individual semen

sample. Variance analysis showed that the mean value was significantly different for SPERM2 in individual samples compared to mixed samples with a difference value of *$P < 2.7$. After analysis of the results of the second DNA methylation marker of semen (SEU2), it was clarified that individual samples had a lower mean with a value of 20.95 than mixed samples which had a mean value of 26.2, illustrating that using the SEU2 marker in individual semen samples is more efficient than in mixed samples. Analysis of variance showed that the mean value for SEU2 marker amplification was significantly different in individual semen samples identification compared to mixed samples with a significant difference value of *$P < 5.8$.

Conclusion

Characterization of body fluids collected from the crime scene plays a significant role in delineating the course of events and helps in the identification of the victim or culprit. Various biochemical techniques have been in use since several decades which are qualitative and destructive in nature. It is very difficult to conclude from a
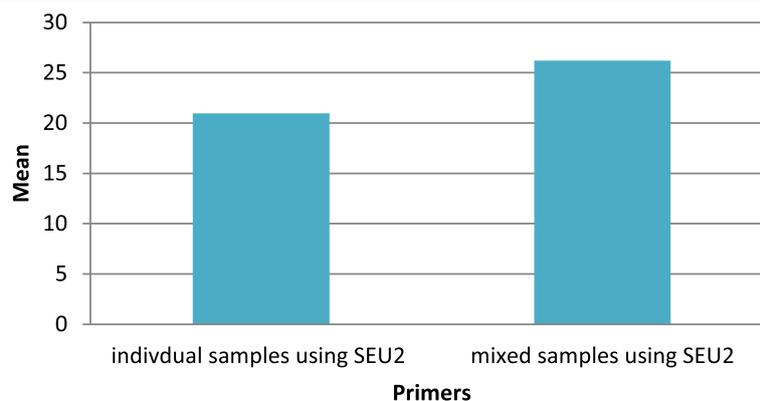

Fig. 5 Comparison between Cq values of SEU2 amplification in individual semen samples and mixed body fluids

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qualitative data especially if the sample amount is very low. Epigenetic markers provide a promising tool in the identification of body fluids and subsequent forensic investigation. Methylation marks do not alter the DNA sequence or base pairing ability. Bisulfite sequencing is a reliable method to study genome-wide methylation patterns. Body fluids recovered from the crime scene play a key role in the construction of a chronological sequence of events. So, the identification of body fluids is an essential step in forensic investigation. It can identify the specific type of body fluid and differentiate between an innocent person and culprit. Previous studies have established characteristic methylation markers for each type of body fluid which still require further supportive studies.

Abbreviations
CpG : A dinucleotide consists of a cytosine followed by a guanine nucleotide; DNA: Deoxyribonucleic acid; DYS14: A gene located on the Y-chromosome within the testis-specific protein Y1 encoding gene; *Hpall*: A restriction enzyme obtained from the microorganism called *Haemophilus parainfluenzae*; RNA: Ribonucleic acid; BLM1-F: Name of the forward primer for the DNA methylation marker in peripheral blood (Cg13763232) on chromosome number 16; BLM 1-R: Name of the reverse primer for the DNA methylation marker in peripheral blood (Cg13763232) on chromosome number 16; MENS1-F: Name of the forward primer for the DNA methylation marker in menstrual blood (Cg09696411) on chromosome number 12; MENS1-R: Name of the reverse primer for the DNA methylation marker in menstrual blood (Cg09696411) on chromosome number 12; SPERM2-F: Name of the forward primer for the DNA methylation marker in semen (Cg05656364) on chromosome number 2; SPERM2-R: Name of the reverse primer for the DNA methylation marker in semen (Cg05656364) on chromosome number 2; SEU2-F: Name of the forward primer for the non-methylated marker in semen (Cg11768416) on chromosome number 5; SEU2-R: Name of the forward primer for the non-methylated marker in semen (Cg11768416) on chromosome number 5

Authors' contributions
R.G.: Creating research idea and design as well as being the main supervisor. L.N.: Supervision, guidance, and editing. J.J.: Performing practical work and writing main text. All authors have read and approved the manuscript

Availability of data and materials
Please contact the author for data requests.

Declarations

Ethics approval and consent to participate
Written informed consents were obtained from all participants in the research which included all the basic information that they need to know about the research project. The research was approved by the University of Modern Sciences Ethical Committee.
The Ethics committee's reference number is not available.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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References
An J, Choi A, Shin K, Yang W, Lee H (2012) DNA methylation-specific multiplex assays for body fluid identification. Int J Legal Med 127(1):35–43

Buckleton J (2009) Validation issues around DNA typing of low level DNA. Forensic Sci Int Genet 3(4):255–260

Cai L (2005) Analytical methods for DNA methylation: methylation-specific PCR and bisulfite sequencing. Newslett Japan Soc Comparative Endocrinol 119: 17–22

Cottrell, S. (2004). A real-time PCR assay for DNA-methylation using methylation-specific blockers. Nucleic Acids Res, 32(1), pp.10e-10.

Forat S, Huettel B, Reinhardt R, Fimmers R, Haidl G, Denschlag D, Olek K (2016) Correction: methylation markers for the identification of body fluids and tissues from forensic trace evidence. PLoS One 11(5):e0156472

Gaydos L, Wang W, Strome S (2014) H3K27me and PRC2 transmit a memory of repression across generations and during development. Science 345(6203): 1515–1518

Gomes J, Kohlmeier F, Schneider P (2011) Genetic markers for body fluid and tissue identification in forensics. Forensic Sci Int 3(1):e469–e470

Hanson E, Ballantyne J (2013) Multiplex high resolution melt (HRM) messenger RNA profiling assays for body fluid identification. Forensic Sci Int 4(1):e125–e126

Jacinto F, Ballestar E, Esteller M (2008) Methyl-DNA immunoprecipitation (MeDIP): hunting down the DNA methylome. BioTechniques 44(1):35–43

Jones B (2014) DNA methylation: switching phenotypes with epialleles. Nat Rev Genet 15(9):572–572

Kader F, Ghai M, Olaniran AO (2019). Characterization of DNA methylation-based markers for human body fluid identification in forensics: a critical review. Int J Legal Med, pp. 1–20 https://doi.org/https://doi.org/10.1007/s00414-019-02181-3.

Lee H, An J, Jung S, Oh Y, Lee E, Choi A, Yang W, Shin K (2015) Genome-wide methylation profiling and a multiplex construction for the identification of body fluids using epigenetic markers. Forensic Sci Int Genet 17:17–24

Nazarenko MS, Markov AV, Lebedev IN, Freidin MB, Sleptcov AA, Koroleva IA, Puzyrev VP (2015) A comparison of genome-wide DNA methylation patterns between different vascular tissues from patients with coronary heart disease. PLoS One 10(4). https://doi.org/10.1371/journal.pone.0122601

Park J, Kwon O, Kim J, Yoo H, Lee H, Woo K, Kim S, Lee S, Kim Y (2014) Identification of body fluid-specific DNA methylation markers for use in forensic science. Forensic Sci Int Genet 13:147–153

Shen L, Waterland RA (2007) Methods of DNA methylation analysis. Curr Opin Clin Nutr Metabol Care 10(5):576–581. https://doi.org/10.1097/mco.0b013e3282bf6f43

Stower H (2012) Epigenetics: dynamic DNA methylation. Nat Rev Genet 13:75

Suzuki M, Greally J (2010) DNA methylation profiling using Hpall tiny fragment enrichment by ligation-mediated PCR (HELP). Methods 52(3):218–222

Tost J, Gut I (2007) DNA methylation analysis by pyrosequencing. Nat Protoc 2(9): 2265–2275

Varriale A, Bernardi G (2010) Distribution of DNA methylation , CpGs and CpG islands in human isochores. 95(1):25–8. https://doi.org/10.1016/j.ygeno.2009.09.006

Vidaki A, Giangasparo F, Court DS (2016) Discovery of potential DNA methylation markers for forensic tissue identification using bisulphite pyrosequencing. Electrophoresis 37(21):2767–2779. https://doi.org/10.1002/elps.201600261

Vidaki A, Kayser M (2018) Recent progress, methods and perspectives in forensic epigenetics. Forensic Sc Int Genet 37:180–195

Virkler K, Lednev I (2009) Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene. Forensic Sci Int 188(1-3):1–17

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