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BIOTECHNOLOGICAL APPLICATIONS IN FORENSICS: QUESTIONING THE EFFECT OF SUN LIGHT CONDITIONS ON DNA INTEGRITY EXTRACTED FROM BLOOD AND HAIR SAMPLES

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Abstract
Biotechnology is deemed to be one of the leading sciences by the fact that is being related to many beneficial applications in our life. One of these applications, is the utilizing the biotechnology in forensic science, which is popularly known as forensic biotechnology, additionally, as the science of biotechnology depends primarily on DNA extraction, it has a vital role in arresting offenders and protecting society. In forensic biotechnology, we deal directly with vital biological evidence such as blood, semen, hair, and various tissues, resulting in imaging of the DNA fingerprint that in the end, leads to capturing the offenders. In conducting our research, we have done several techniques to examine the effect of sunlight conditions on DNA extracts obtained from both, blood and hair samples. In the sake of quantification of the DNA extracts, Nano Drop spectrophotometer device was used to obtain concentrations, 260/280 and 260/230 ratios of the samples, values of 260/280 ratio for fresh blood samples, blood samples kept under sun light conditions and blood samples stored in shadow conditions was 2.342, 1260 and 1.355 respectively. Furthermore, integrity of the DNA samples was determined using agarose gel electrophoresis, random amplified polymorphic DNA and STR analysis, results showed poor and faint bands that referred to all DNA extracts obtained from samples whether exposed to sunlight or not, caused by the low quality and quantity of the DNA samples obtained. Although several former researches proved the effect of the sunlight conditions on DNA, the low quality compared to control samples made it difficult to observe that effect.

Keywords: Forensic science, DNA, Forensic biotechnology.

1. Introduction

A novel branch of forensic science has been formed as a result of the application of biotechnological techniques in the forensic investigation, and this branch was named as "Forensic Biotechnology". Moreover, DNA fingerprinting which is related to forensic biotechnology is considered as a crucial component in identifying DNA that obtained from a variety of biological samples, such as hair, blood or biological fluids samples that collected at the crime site. The forensic biotechnologist job can be defined as the ability of linking a suspect to the crime site or aiding in identifying an unknown person

[1]. Formerly, certain proteins and markers were utilized to complete the characterization of the biological specimens taken from the crime scene. Several markers were employed to settle the results of the identification procedures, like blood group antigens and serum proteins. The disadvantage of using such markers was having many obstacles in their employment, like poor stability, confined activity, low polymorphism and low range of error-free detection methods. On the other hand, using DNA markers offer more precise results and higher recognition power for the forensic testing [2]. Based on what mentioned, a variety of biological samples can yield DNA, like hair, blood, semen and saliva. In biological research, blood has gained more remarkable importance than the other types of samples. Forensic investigations, hematology, and Biochemistry have incorporated blood as an integral part of their studies. The presence of nucleated white blood cells makes it an important source of genomic DNA. Additionally, DNA isolation from blood has been the subject of numerous protocols that have been published [3- 6]. Enzymes and organic solvents are employed in some published protocols to produce high-quality DNA without the use of PCR inhibitors, while others involve a salting out procedure designed to boost DNA yields [7, 8]. There are protocols that are both costly and time-consuming, while others harm the quality of DNA [9- 12]. The ability of the extracts to be used for long-term DNA-banking is an important aspect of DNA extraction protocols and advanced genotyping analysis. The storage of DNA extracts is required until all samples are collected, which can vary depending on the study. Furthermore, there is a heightened interest in the creation of DNA banks due to the strenuous procedures of sample collection and extraction. To measure the integrity of the DNA extracts, several storage examinations are carried out by the DNA Bank Network, due to the probability of damaging the DNA by the presence of secondary compounds and heavy metal ions, which have the ability to form highly reactive intermediates [13, 14]. DNA synthesis in mammalian cells can be inhibited by ultraviolet irradiation (UV), but there is no mechanism that was clearly stated for this inhibition [15]. It has been revealed that cells which were subjected to UV light radiation were having a higher probability to form some sort of DNA lesions; the two most common lesions were the cyclobutane pyrimidine dimer and the 6-4 pyrimidine pyrimidone photoproduct at adjacent pyrimidine. Furthermore, DNA lesions result in cell death and mutations if the cell failed to regain its normal state [16].

This research was conducted to study the effect of light on the quality of DNA extracts that have been obtained from different types of specimens, like hair and blood.

2. Materials and methods

2.1. Sample collection

2.1.1. Blood sampling and DNA extraction

EDTA-containing vacutainer tubes were used to gather samples from 30 healthy individuals at random manner, then half of the collected samples was kept under light conditions, while the other half is stored under shadow conditions (Figure 1). The sample collection was taken place at Taif, Saudi Arabia. A document of consent has been filled by each individual to confirm about being a part of the study. Medical history was taken from each participant to make sure that nothing of the procedures will have a negative effect on their health. History has shown that no one of the participants has any diseases, which could form an obstacle to continue the process. The study was approved to be done by the Human Ethics Committee of Taif University, Saudi Arabia and was performed in a manner that agrees with the Helsinki Declaration of 1975. At first, DNA extraction procedures took place by transferring an amount equals to 500 μL of blood sample to an eppendorf tube, then by centrifugation plasma was aspirated out at 2664 RCF for duration of 7 minutes at 4°C, after that, 1 mL of RLB was added to the precipitate, mixed, kept at room temperature for 2 minutes and the supernatant was discarded. Furthermore, the pellet was mixed with pre-warmed DNA extraction buffer, 10% SDS, and B-Mercaptoethanol, and then added with gentle mixing. At 56-60°C, the mixture was left to incubate for one hour. After incubation, the mixture was shaken thoroughly before being added 500 mL of Chloroform: isoamylalcohol (24:1). Centrifugation was done for 12 minutes at 10 656 RCF at 4°C. Furthermore, the supernatant was transferred to a new sterilized centrifuge tube

that was containing chilled ethanol. Fine white threads appeared in the solution after shaking the tube for some time. The sample tube was stored at −20°C for 20 minutes. Furthermore, the sample underwent centrifugation at 10 656 RCF for 12 minutes at 4°C, and the supernatant was removed. At the final step, the supernatant was discarded, the pellet was then dissolved overnight in 100 µL of TE buffer and the DNA solution was then stored at −20°C for future use [17].

2.1.2. Hair sampling and DNA extraction

Using diverse lengths of hair shafts, various types of samples were utilized for DNA extraction. Hair shaft samples were made up from hair of 30 candidates, which were vary in their characteristics from being thick or thin, then 15 samples were stored under light conditions, whereas the other 15 were kept under shadow conditions (Figure 2). Before DNA extraction began, hair samples have been decontaminated using a solution of 10% sodium hypochlorite. Hair shafts have been immersed in bleach for nearly 5 min at room temperature; in addition to that, they were exposed to rinsing twice using sterile double-distilled water. As a final step, samples were kept to dry at room temperature. The reproducibility of all samples was tested by analyzing them in triplicate. The PrepFiler BTA Express TM protocol and the Auto Mate Express TM apparatus (Applied Biosystems) were utilized to extract DNA from hair shafts. Different incubation times in the lysis step were tested in order to optimize the protocol [18].

2.2. Estimation of DNA concentration using Nano Drop Spectrophotometer

DNA quantification was done using THERMO Scientific Nano Drop device, the process was involved a respectable number of steps to obtain the value of DNA concentration. Firstly, upper and lower optical surfaces of the spectrophotometer sample retention system were cleaned using deionized water. Secondly, from the NanoDrop software, nucleic acid application was selected, additionally, a calibrated pipettor was used to perform a blank measurement by dispensing 1 µL of buffer onto the lower optical surface, then, lever arm was lowered and "Blank" was selected in nucleic acid application. Moreover, sample was placed onto the lower pedestal, "Measure" was selected in the application and spectral image was assessed for determining sample quality. Lastly, to get an accurate assay of samples quality, 260/280 or 260/230 ratios were analyzed in combination with overall spectral quality [19].

2.3. Assessment of DNA purity and integrity

2.3.1. Using Random amplified polymorphic DNA

Polymerase Chain Reaction using random short synthetic primers was carried out on the extracted DNA samples in order to check the proficiency of the extracted DNA. Unique primers have been employed for the sake of amplification process completion. Primers used were supplied from Metabion (Germany). The target sequence was amplified with initial heating at 96ºC for 5 minutes, followed by 40 cycles of denaturing at 96ºC for 30 seconds. In addition to that, annealing step was done by using a temperature of 58ºC for 30 seconds extension at 72ºC for 40 seconds, followed by a final elongation step 72ºC for 5 minutes (Table 1).

Table 1. Target gene, primer sequence and conditions of PCR cycles

Target gene	Primers sequences	Amplified segment (bp)	Prim. Den.	Amplification (35 cycles)			Final extension
				Sec. den.	Ann.	Ext.	
B-Actin	5`-TGCTATCCCTGTACGCCTCT-3`	130	96˚C 5 min.	96˚C 30 sec.	58˚C 30 sec.	72˚C 40 sec.	72˚C 5 min.
	5`-CGTCATACTCCTGCTTGCTG-3`						

2.3.2. Using short tandem repeat (STR) analysis

All experiments were aided by using the primer sets of the Identifiler® PCR Amplification Kit. MicroAmp reaction tubes were used to perform the PCR amplifications, which involved 1 ng of control DNA 007 in a reaction volume of 25 ml within the PCR Systems 9700 with a silver block or the Veriti1 thermal cycler (Applied Biosystems). The AmpliTaq Gold enzyme was used in the experiments and thermal cycling conditions included 11 minutes at 95°C followed by 28 1 minute cycles at 94°C, 59 ºC for one minute, 72 ºC for one minute, and final extension at 64 ºC for 40 min. The manufacturer's recommendations were followed for separation and detection of PCR products on an ABI PRISM1 3100 Genetic Analyzer. The mixture of 1 ml of PCR product, 8.7 ml of Hi-Di Formamide, and 0.3 ml of Gene Scan TM 500 LIZ1 size standard was done in a way that included mixing. The analysis of data was done using Gene Mapper ID v3.2 software [20].

2.3.3. Using agarose gel electrophoresis

One percent agarose gel electrophoresis was implemented to assess the integrity of DNA extracts, which have been obtained from different sources. To prepare the gel, we add an appropriate amount of agarose powder to the TBE in a Flask and heat it in a water bath to 100°C (boiling temperature) until the powder dissolves well. Then we cool the solution to a temperature of 60 °C, and then add ethidium bromide to it. The comb is placed at a height of 0.5 - 1.0 mm above the gel pouring container until it turns into a hole. When adding the agarose gel, we pour the hot gel solution onto the gel pouring bowl. It should be 3 mm to 5 mm thick. Pour it gently so that no air bubbles appear on the surface. Then we wait for the gel to solidify from 30 to 45 minutes at room temperature, and then carefully remove the comb. Mixing DNA samples with loading buffer and using micropipette Samples are placed in the hole in the electrophoresis chamber. A sufficient amount of TPE is placed in the electrophoresis chamber until it rises 1 mm above the gel. We close the cover, and then put the electrodes, heath migrates DNA towards the red electrode, and to indicate that the device is working; bubbles appear at the black electrode. After completion, the current is turned off, the electrodes are removed, and the gel container is raised using device UV Transilluminator DNA samples are seen stained with ethidium bromide using a wavelength of 300-350 nm [21].

3. Results

3.1. Quantification of DNA using NanoDrop device

Through utilization of THERMO Scientific NanoDrop device we have obtained a number of parameters, like 260/280 ratio, 260/230 ratio and DNA concentrations (Figure 3). The 260/280 ratio for fresh blood samples, blood samples kept under sun light conditions and blood samples stored in shadow conditions was 2.342, 1260 and 1.355 respectively. According to results, the DNA quality was not that good as expected to be.

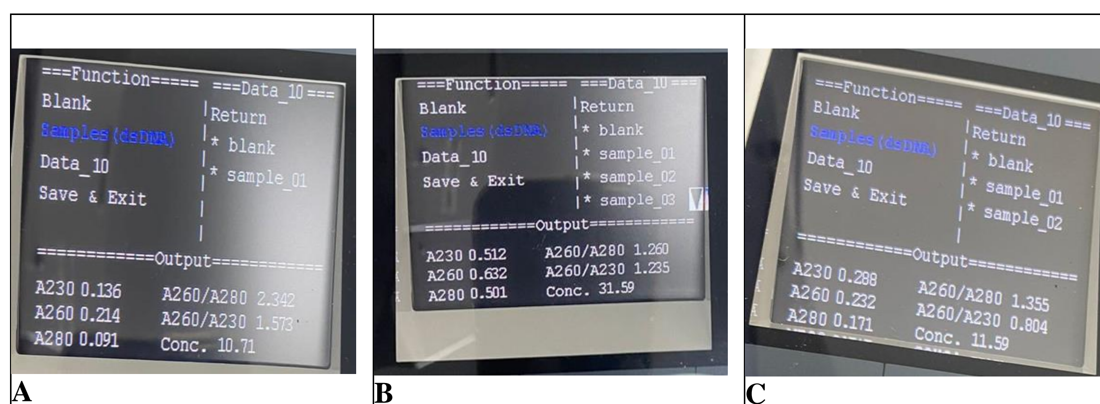

Figure 3. (A) Fresh Blood Samples (control) DNA concentration. (B) DNA concentration extracted from Blood samples that placed under the sun light conditions. (C) DNA concentration extracted from Blood samples, which placed in the shadow conditions**.**

3.2. Assessment of DNA integrity

3.2.1. Random amplified polymorphic DNA

B-actin gene was used as a control since it expresses in almost all cells and known as a constitutive gene. Moreover, the band represents B-actin is obvious and shiny, indicating a good DNA quality (Figure 4). In addition, band 3 in Figure 4 point out DNA extracted from fresh Blood samples and it appears faint, but still clear. On the other hand, the band related to DNA extracted from blood sample under sun light conditions came out as less clear and very week, while the band related to DNA extracted from blood samples in the shadow is clear but not in the expected size.

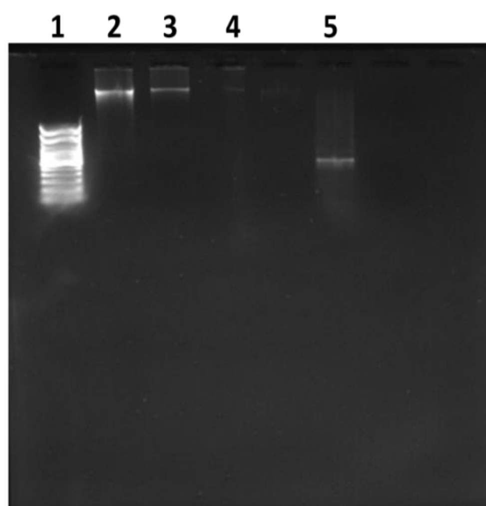

Figure 4. (1) DNA marker (DNA ladder, 100 bp). (2) B-actin gene. (3) DNA extracted from fresh blood samples. (4) DNA extracted from blood samples under sun light. (5) DNA extracted from blood samples in the shadow.

3.2.2. Short Tandem repeat (STR) analysis

Short Tandem Repeat (STR) profiling was performed on DNA extracted from fresh blood samples, DNA extracted from blood samples under sun light and DNA extracted from blood samples kept in shadow conditions (Figure 5). Result reveals that several unspecific DNA bands were appeared; in addition to that, 12 to 16 lines present unclear and uncertain DNA bands.

Figure 5. (1) DNA marker (DNA ladder, 100 bp). (2, 3) DNA extracted from fresh blood samples. (4-10) DNA extracted from blood samples under sun light. (11-16) DNA extracted from blood samples in the shadow.

3.2.3. Electrophoresis

The quality of all DNA samples collected from different sources and different conditions during this study were determined using gel electrophoresis technique, which enables researchers to know the quality of genomic DNA. It can be seen that the bands obtained were faint and weak comparing with B-actin and DNA samples, which used as a controls (Figure 6).

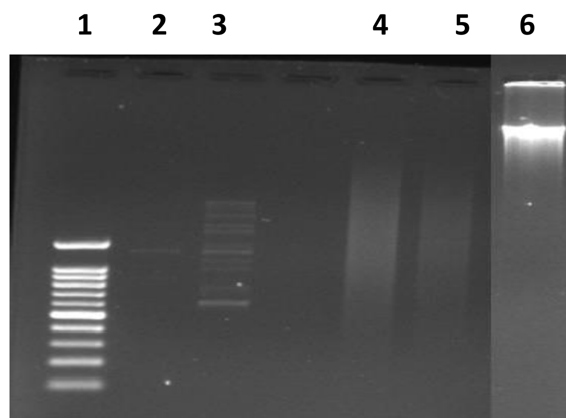

Figure 6. (1) DNA marker (DNA ladder, 100 bp). (2) B-actin DNA. (3) DNA extracted from fresh blood samples. (4) DNA extracted from blood samples under sun light. (5) DNA extracted from blood samples in the shadow. (6) DNA sample as a control.

4. Discussion

Biotechnology approaches are becoming more important in criminal investigations when it comes to forensic analysis of biological evidence. For example, by implementation of recombinant DNA techniques, which are used in biotechnology, we've discovered several DNA polymorphisms, and some of them are crucial in analyzing biological materials obtained from crime scenes [22]. In this research, we have evaluated the effect of sunlight conditions on quality and quantity on DNA extracts obtained from both, blood and hair samples. It is clear from the study that the implementation of the extraction method mentioned was not of highly feasible since DNA concentration obtained from samples was low, resulting in the difficulty of determining the effect of sunlight on blood samples that have different climatic conditions, despite the effect of sunlight on vital samples such as hair, semen, tissues in general, and blood samples in particular has been scientifically proven in former studies [23]. Understanding the impact of the denaturation process and the physical changes that occur to blood when exposed to sunlight conditions could lead to the development of new forensic investigative tools, such as the use of reflected infrared photography to improve bloodstain recording. When subjected to heat > 200 °C, blood denaturation, specifically changes in the hemoglobin state from oxyhemoglobin to methemoglobin [24]. In regard to the assessment of the DNA extracts in this study, when examining the DNA with gel electrophoresis, it became apparent that the resulting DNA band was very weak and faint compared to the DNA control. The reason for this might be poor concentration of the extracted DNA. Furthermore, PCR using both short random primers and Tandem primers has been performed on DNA extracts to evaluate the integrity. Short primers and STR profiling indicated that, the bands resulting from the examination of the gel electrophoresis were weak and unclear, and this caused by the weak concentration and quality of the extracted DNA.

5. Conclusion

The extraction of DNA from vital evidence, such as blood or hair, which commonly collected from the crime scene, is one of the most important evidences that used in forensic sciences since it provides such a tremendous amount of information in the investigation. In addition, these samples and Biological evidences might be affected by various physical factors such as light. In this study, the effect of sunlight conditions on DNA yield from both, blood and hair samples were examined. The

evaluation involved the using of NanoDrop spectrophotometer to quantify the DNA extracts, agarose gel electrophoresis and PCR implementation through both, random amplified polymorphic DNA and STR analysis to determine the integrity of the DNA extracts after being exposed to both, sunlight and shadow conditions. Compared to control sample, the results of these techniques revealed the low quality and quantity of the DNA extracts obtained from blood and hair samples, which represented by the faint bands showed in PCR techniques and the values of low concentration obtained from NanoDrop spectrophotometer. The poor integrity of the DNA extracts made it hard to evaluate the effect of sunlight conditions.

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Data Availability Statement
The Material section of the article contains the study's original contributions; any further questions should be directed to the relevant author..

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