# Analysis of Blood Spot in the Crime Scene in Relation to the Environmental Parameters

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## Abstract

This study was designed to evaluate the analysis of blood samples in crime scene under different environmental conditions. Samples are divided into four main groups depending on the source of bloodspot: Soil, Stone, Ceramic, and Water sample. Each group consisted of two sub-groups depending on weather temperature in Iraq in winter and summer: 8 °C groups and 45 °C groups. Each group consisted of 32 samples. Samples were collected after different time (0, 2, 6, and 24) hours.

The results of the current study showed variation in the ability to extract DNA depending on the surface type. In addition, DNA can be extracted from bloodstains on soil, gravel, ceramics, and water when extracted immediately after exposure to the surface. The difference in the ability to extract DNA from bloodstains on different surfaces after two hours at 8 °C temperature, where the success rate of extraction is (25, 7.5, 100, and 100%) for soil, stone, ceramics, and water samples Respectively. The ability rate to extract DNA after two hours at 45 °C temperature, where (25, 7.5, 87.5, and 100%) for soil, gravel, ceramics, and water samples respectively. The ability rate to extract DNA after six hours at 8 °C temperature, where (0, 12.5, 37.5, and 100%) for soil, gravel, ceramics, and water samples respectively, while it was (0, 12.5, 25, and 100%) for six hours samples at 8 °C temperature. After 24 hours, only water samples showed the ability to extract DNA from all samples, while no DNA was obtained from the rest of the samples.

PCR amplification of the *FIF2AK3* gene showed products when the DNA extracted immediately after their adhesion to the surface of the soil while showing no products for other age groups. Also, the PCR products can be obtained when DNA isolate immediately after exposure to the stone surface, whereas the rate PCR amplification products of DNA isolated after two hours was (37.5%) for both groups 8 °C and 45 °C., while other age groups showing not products.

The agarose gel electrophoresis of PCR products showed the possibility of *FIF2AK3* gene amplification when blood samples were taken immediately after exposure to the surface, whereas the ability to obtain amplification products was (100 and 87.5%) for groups 8 °C and 45 °C respectively. No products were obtained after 6 and 24 hours. In water samples, all isolated DNA gave the product.

## Introduction

The term “forensic genetics” Refers to the use of human DNA applications in crime investigation (1). The use of Deoxyribonucleic acid (DNA) as evidence of its origin has considered a revolution in the field of forensic. The accessibility of improved technologies has now made DNA analysis a critical and part of forensic science (2,3). DNA profiling has a high discriminatory power, which made it particularly influential in forensic science. By using of a number of genetic markers, DNA profiling has the ability to differentiate between individuals, which gave each individual a distinctive, possibly unique, DNA profile (4).

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DNA evidence can support an investigation along with other evidence to allow the possibility of including or excluding a suspect from their presence in a crime. DNA discrimination power makes it a powerful tool that can assist an investigation as evidence to be presented in court (5). Evidence including body fluid (such as blood, saliva, semen, and sweat), tissues, for example, teeth, skin, hair roots, and bones can all be used to produce a DNA profile (6). The natural physical environment such as temperature, humidity, and ultra-violet radiation, can affect the ability to recover DNA from samples. These effects depend upon the location and climatic conditions. A number of environmental influences have a direct impact on DNA degradation. After all, the high temperatures and ultraviolet (UV) is one of the most important factors that increase the degradation of DNA (7).

Limited studies have addressed the effects on the environment of DNA spots resulting from the body fluids may be due to the fact that there is a difference in large climates between different geographical locations that make this measure scientifically impossible (8)

## Materials and Methods

Samples of the study collected randomly from the human blood for the period from 1/2/2019 to 15/6/2019, the samples have consisted of deferent blood groups with different environmental conditions. One ml of blood used to simulates the crime scene. Samples divided into four main groups depending on the source of bloodspot: Soil, Ceramic, Stone, and Water sample. Each one of the mains consisted of two sub-groups depending on weather temperature: 8 C group and 45 C group. Each group consisted of 32 samples with different blood groups. DNA extracted from the samples immediately, after tow houres, after 6 hours, and after 24 hours.

Genomic DNA extracted from the blood of samples according to the extraction method adopted by the supplied company.

Electrophoresis has been done to determine DNA pieces after the process of extraction or to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the Agarose gel . Than we used nanodrop to determination of DNA concentration.

The primers where design by primer 3 plus program. The primers were lyophilized, they dissolved in the free ddH2O to give a final concentration of 100 pmol/µl as stock solution and keep a stock at -20 to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free ddH2O water to reach a final volume 100 µl.

| Primer | Sequence | Tm (°C) | Product size |
|---|---|---|---|
| F | 5'-AGCTCCTATAGTAACCTCTTCTTGAACTCACTTG- 3' | 68.6 | 429 bp |
| R | 5'-GCTTTCACGGTCTCGGTCCCACTG- 3' | | |

## Results and Discussion

DNA was extracted from blood spot in different invironmental condition (Soil, stone, ceramic, and water) at (8 °C and 45 C°). Each sample's source was composed of 32 samples, with samples age group (0, 2, 6, and 24 hours). The concentration of the extracted DNA cheeked by using a nanodrop device.

The DNA concentration of the Soils samples at 8 °C ranged from 32.3 to 67.1 ng/µl with average 51.09 ng/µl, while the DNA concentration of the samples that taken at 45° C ranged from 22.3 to 54.3 ng/µl with average 38.24 ng/µl. The DNA concentration of stone samples at 8 °C were ranged 32-86.9 ng/µl with average 60.6 ng/µl, while the DNA concentration of 45 °C samples was ranged 23-65 ng/µl with average 46.8 ng/µl. The DNA concentration of 8 °C samples was ranged 39 to 77 ng/µl with average 60.6 ng/µl, while the DNA concentration of 45 °C samples was ranged 32 to 66 ng/µl with average 49.2 ng/µl. The DNA concentration of water samples 8

°C was ranged 23 to 70 ng/µl with average 42.7 ng/µl, while the DNA concentration at 45 °C was ranged 20 to 74 ng/µl with average 41.4 ng/µl.

Agarose Gel electrophoresis of extracted DNA from soil samples showed bands in 10 samples out of 32 samples in each 8 °C and 45 °C. Eight band came from DNA extracted from immediately age group, 2 bands from 2 hours age group, while none of the other age group samples gave any bands. Stone samples showed 12 bands of 32 samples for 8 °C samples. The band composed of eight bands from zero age group, three bands from 2-hour age group, and one band from 6-hour age group. The 45 °C samples gave also 12 bands distributed as eight bands for the zero-age group, 3 bands for (2 hours) age group, and one band for (6 hours) age group. Agarose Gel electrophoresis of extracted DNA showed 19 bands of 8 °C samples. The band composed of 8 bands from zero age group, 8 bands from 2 hour age group, and 3 bands from 6 hour age group. The 45 °C samples give 17 bands distributed as 8, 7, and 2 bands for 0, 2, and 6 hour age group. Agarose Gel electrophoresis of extracted DNA products showed the appearance of all bands for 8 °Cand 45 °Csamples fig. (1).

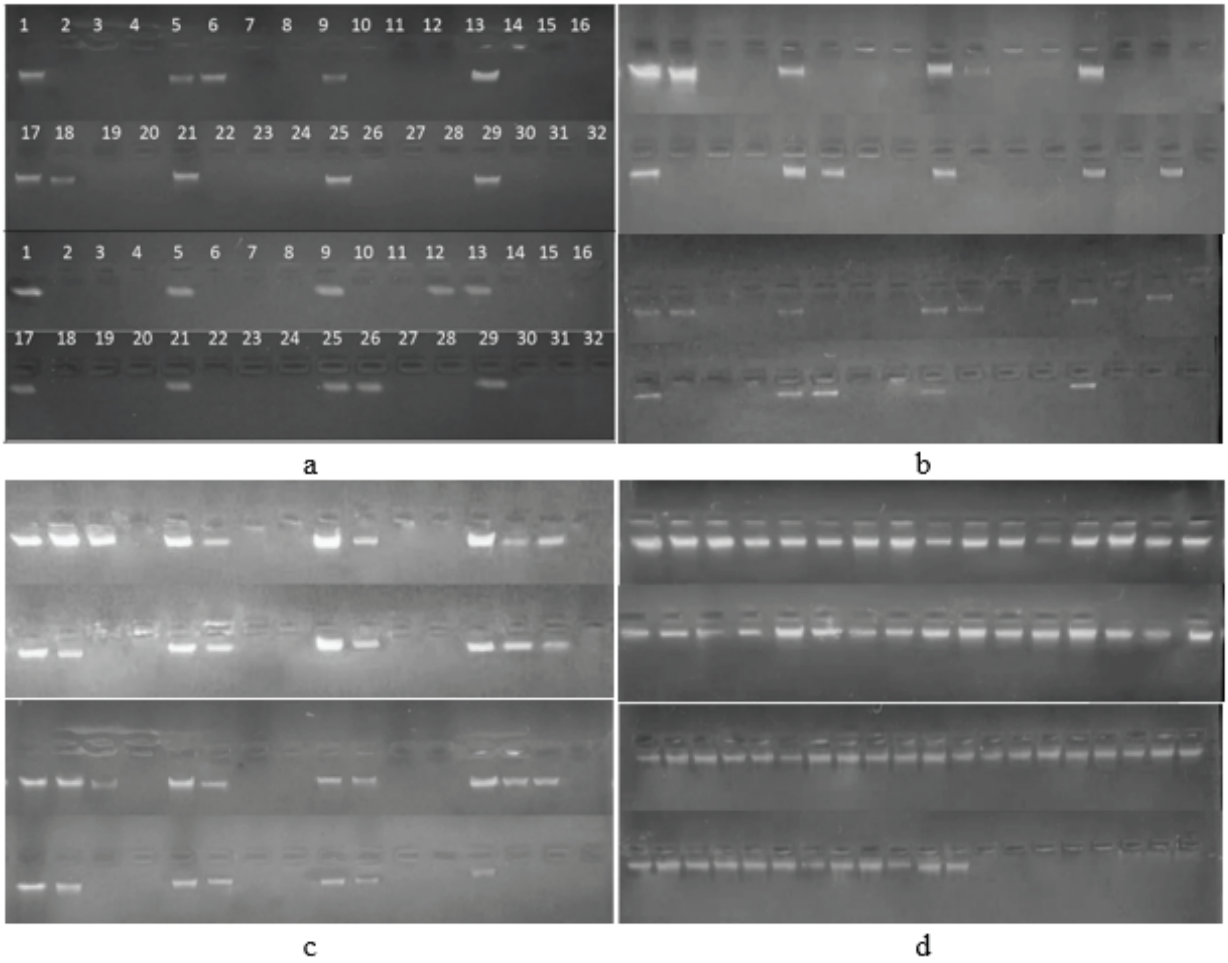


**Fig (1) Agarose gel electrophoresis of genomic DNA extracted from blood spot on 1.5% agarose at 50v. for 2 hr (a) Soil sampes at 8 °C and 45 °C (b) Stone sampes at 8 °C and 45 °C (A) Ceramic sampes at 8 °C and 45 °C (A) Water sampes at 8 °C and 45 °C group**

Agarose gel electrophoresis of PCR product by using specific primers for amplification of gene for 30 samples of blood spot taken from the soil at 8 °Cand 45 °C showed 8 bands at the expected site in both (429 bp). All bands represent immediately age group. Ceramic samples at 8 °C and 45 °C showed bands in 11 samples at the expected site (429bp) in both. The distribution of 8 °C and 45 °C samples depending on age group was eight bands for the zero-age group and three bands for 2 hours. Stone samples showed bands for 16 samples in both 8 °C and 45 °C group at

the expected site. Depending on samples age, the distribution of 8 °C group was eight, and 8) bands for (zero and 2) hour age groups respectively, while the distribution of 45 °C was also (eight and 8) bands for (zero and 2) hours age groups respectively. In water samples, they gave product in expected site for all samples.

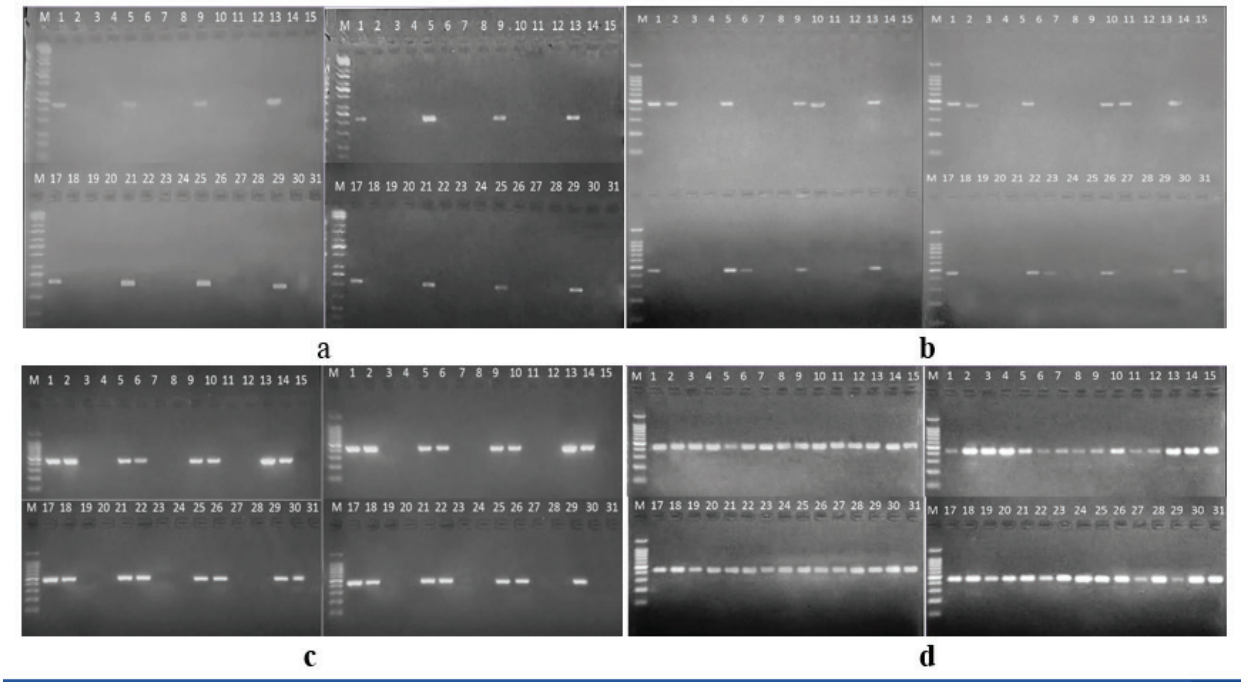


**Figure (4-5) Agarose gel electrophoresis of PCR product for amplification of gene for blood spot on 1.5% agarose at 50 v. for 1 hour (a) Soil sampes at 8 °C and 45 °C (b) Stone sampes at 8 °C and 45 °C (A) Ceramic sampes at 8 °C and 45 °C (A) Water sampes at 8 °C and 45 °C group**

## Discussion

In the present study, we used two different temperatures (8 ° C and 45 ° C) to simulation the weather in Iraq. We used four environmental sources to stimulate the crime scene.

Biological evidence may expose to various environmental factors. These factors play crucial roles in DNA degradation (9). Environmental conditions such as heat, humidity, chemistry, microorganisms (bacteria and fungi), etc. can be effective on the stability of DNA for survival (10).

The DNA extracted from the blood spot affected by the nature of the surface on Moreover, the concentration and purity of the extracted DNA influenced by the surface factor in it (11). A study showed that the concentration of DNA is higher and the possibility of extraction is stronger on smooth surface comparison with non-smooth surfaces (12)

Extracting DNA from biological evidence found in the soil has a high failure rate. This may be due to the soil contains many microbes that can cause DNA destruction and digestion. Moreover, containing soils of different chemical compounds can do the same action (12). The presence of an inhibitory substance called humic acid in natural soils may cause DNA degradation (13).

The Hem and humic acid act as inhibitors Exposure to mild (moderate) inhibitors reduce the concentration of DNA (14).

The reaction of moderate enzymes also plays a role in DNA degradation when undergoing external environmental conditions. When cells die, the internal enzymes are activated, which leads to DNA degradation by an endonuclease or exogenous which released by the microorganisms that exist in the surrounding environment turned to mononucleotides (10).

This is consistent with the current study, where blood spot on soil has been affected by two inhibitors:

humic acid from soil and hem from RBC, in edition to microorganisims. his lead to DNA degradation in most of the samples. Stone and ceramic samples were less degradations from soil samples this may due to less affected inhibitors and the nature of its surface. In water samples, the appearance of all samples may refer to that water reduce the inhibitory effect of the hemoglobin.

Many studies showed that temperature plays a big role in DNA degradation (9). Another study mentions that temperatures are known to have the ability to reduce the construction of DNA (6).

This agreed with the current study which found that DNA constriction affected by weather temperature in soil, stone and ceramic, while it shows no effect in the water. This may due to water specific heat.

The presence of an inhibitory substance called humic acid in natural soils; this substance is extracted with DNA extraction and inhibits the possibility of DNA profile by binding to DNA molecular components (13)

The natural soil contains many PCR inhibitors. However, the main inhibitor responsible for the high rate of failure to identify DNA is humic acid; this substance is extracted with DNA extraction and inhibits the possibility of DNA profile by binding to DNA molecular components. Humic acid consists naturally through the decomposition of plant and animal remains. Humic acid Dominates the grassland soil (13,15).

Humic acid has chemical-physical properties similar to double-strand DNA and is co-extracted with DNA, either by binding with components of the extraction system (silica) or by binding to the DNA molecule itself (16). HA linking with Most of PCR components and inhibits DNA amplification (15,16), which not only directly effects on DNA profile, but also indirectly by affecting the accuracy of the quantification of DNA using PCR (13).

Forensic samples contain chemicals that can reduce or inhibit amplification using polymerase chain reaction (PCR). The most obvious inhibitor is (hem) (17), and dyes found in clothing and humic acid from soil samples and calcium from the bone (18). These substances are extracted with the DNA product and can affect all the contents of the polymerase chain reaction including the DNA template, nucleotides, amplification primers, $Mg^{+2}$, And polymerase enzymes. Although the inhibitory effect of these substances is known, the inhibitory mechanism is unknown (19). Exposure to severe inhibitors leads to loss of alleles (14)

In a study by Wu and co-workers, the characteristics of the RBC membrane are influenced by the type of substance-exposed to the blood (18).

This agreed with our result where PCR amplification did not give any band in 2 and 2 samples at 8 C and 45 C respectively. All samples were 2 hours of age group. PCR amplification did not give any band in 3 and 3 samples at 8 C and 45 C respectively. All samples were 6 hours age group. This may refer that soil has a faster inhibitory rate than ceramic and stone. Our study shows no Impact of water on DNA stability this may due to the percentage of blood volume to water is low.

## Conclusion

DNA is affected by different surface in which blood stains. Temperature affects the concentration of DNA in exposed blood stains while it does not affect the presence of stains in water. The concentration of DNA is not affected by water extraction and the temperature of the ocean does not affect the DNA in water

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

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