

Mini Review

# Environmental Factors Affecting the Concentration of DNA in Blood and Saliva Stains: A Review

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## Abstract

DNA evidence has now become an essential part of forensic investigations since it offers vital information for person identification and crime resolution. However, the biological material is affected by some environmental factors which may impact the DNA in biological samples. This may affect the correctness and reliability of forensic DNA analysis. This review is related to the influence of various environmental conditions on the stability and degradation of DNA in biological stains including blood and saliva stains. The common factors that affect DNA are temperature, humidity, exposure to sunlight, and type of substrate. The information is crucial to improve forensic DNA analysis and forensic protocol optimization. The DNA stability and integrity in biological materials, such as blood and saliva stains, are indispensable for forensic DNA analysis. Environmental influences, however, significantly affect DNA concentration and may jeopardize forensic analysis. The present review explores various environmental factors for their effect on DNA stability in blood and saliva stains. While DNA degradation is slowed but not completely prevented by low temperatures, it is accelerated by high temperatures. Risks of contamination arise from the promotion of microbial growth and DNA breakdown by humidity. DNA photodamage brought on by sunlight exposure results in strand breakage and cross-linking. DNA stability is also influenced by the type of substrate used; porous surfaces, such as cloth, are better at keeping fluids than non-porous ones, such as glass. Maintaining the integrity of DNA evidence requires an understanding of these variables. The present studies will help to create sophisticated DNA preservation methods for use in forensic DNA examination. The study emphasizes the requirement of improvement in forensic DNA analysis skills, related to the preservation of DNA pieces of evidence and the possible effect of environmental factors.

## Introduction

Forensic DNA analysis has had a tremendous influence on criminal investigations, giving critical evidence for suspect identification, exonerating innocent people, and solving crimes. Biological samples, such as blood and saliva stains, are frequently discovered at crime scenes and can yield vital DNA evidence [1-3]. However, environmental factors that alter the stability and concentration of DNA in these biological stains can jeopardize the accuracy and dependability of forensic DNA analysis. Multiple environmental factors encountered during sample collection, storage, and analysis impact the stability of DNA in biological samples. The main variables that affect DNA integrity are substrate type, temperature, humidity, and solar exposure [4,5]

Forensic scientists must comprehend how these environmental conditions affect DNA evidence integrity preservation in criminal investigations to optimize processes. One important environmental component influencing DNA

stability is temperature. High temperatures hasten the oxidation and hydrolysis processes that break down DNA, resulting in the fragmentation of DNA molecules [6]. On the other hand, low temperatures may not completely stop degradation but can certainly slow it down, which emphasizes how crucial temperature management is while handling and storing samples. The environmental factors lead to DNA degradation and result in low levels of STR peaks. This Environmental degradation can lead to complex DNA lesions and fragmentation [7,8].

In biological stains, exposure to sunshine can seriously harm DNA molecules. Sunlight's Ultraviolet (UV) rays cause strand breakage and cross-linking in DNA due to photodamage. Long-term sun exposure can cause significant DNA deterioration, which makes forensic analysis difficult. Maintaining the integrity of DNA evidence at crime scenes requires preventative measures including limiting sunlight exposure and utilizing UV-protected materials [9].

## More Information

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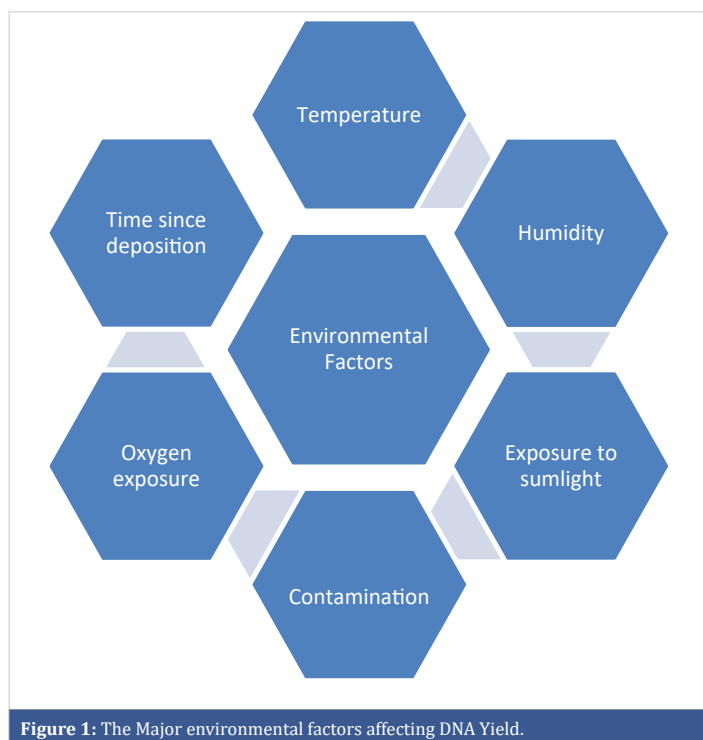


DNA stability is also significantly influenced by humidity levels. High humidity encourages the growth of microorganisms, which raises the possibility of contaminating and degrading DNA. Furthermore, environmental dampness can promote the hydrolysis of DNA, jeopardizing the integrity of DNA even further. Thus, it is essential to maintain proper humidity levels during sample processing and storage to preserve the integrity of DNA evidence [10-12]. In conclusion, the stability and integrity of DNA in blood and saliva stains are greatly impacted by environmental variables, which in turn affects the validity of forensic DNA analysis. Comprehending these variables is imperative in formulating efficacious forensic procedures and guaranteeing the conservation of DNA evidence integrity within criminal inquiries [13].

Forensic scientists can improve the precision and dependability of DNA analysis by regulating temperature, humidity, sunlight exposure, and substrate type, which will ultimately help in the administration of justice [14] (Figure 1).

### Environmental factors affecting the yield of DNA

The stability and integrity of DNA in blood and saliva stains are significantly influenced by environmental variables, which in turn affects the dependability of forensic DNA analysis. These variables include a range of situations that may arise during sample preparation, storage, and examination, all of which may have an impact on the preservation and degradation of DNA [15]. Forensic scientists must have a thorough understanding of environmental influences to create efficient procedures and guarantee the quality of DNA evidence interpretation in criminal investigations. The DNA degraded by environmental factors results in a reduction in the amplification and sequencing process [16].



### Temperature

The stability of DNA is significantly impacted by temperature. High temperatures hasten the oxidation and hydrolysis processes that break down DNA, resulting in DNA molecule fragmentation. Low temperatures, on the other hand, may not completely stop deterioration but can certainly slow it down. To reduce DNA degradation and maintain sample integrity, temperature control must be maintained during sample handling, storage, and transportation [17]. As per the research paper 'Investigation of DNA degradation in forensic blood samples after exposure to different environmental conditions' the DNA gradually degraded as the exposure time at 55°C temperature was increased [18].

In forensic science, temperature has a significant impact on DNA stability, especially when examining biological materials like blood and saliva stains. Through a variety of biochemical and physical mechanisms, temperature affects DNA stability, which in turn affects the accuracy and dependability of DNA analysis [19]. Forensic scientists must comprehend the effects of temperature to create procedures that reduce DNA deterioration and maintain sample integrity. The following are the main ways that temperature influences DNA stability:

**DNA degradation:** The rate at which DNA degrades is significantly accelerated by temperature. Elevated temperatures can accelerate DNA molecule hydrolysis, which can result in phosphodiester bond breaking and DNA strand fragmentation. This process breaks down DNA into smaller bits and is most noticeable at high temperatures. Because DNA fragmentation alters the quality and amount of DNA accessible for profiling, it can make forensic analysis more difficult [19].

**Denaturation:** When hydrogen bonds between complementary base pairs are broken, the DNA double helix separates into its constituent strands, a process known as denaturation. Higher temperatures encourage denaturation of DNA molecules, which in turn affects the stability of DNA duplexes. Denaturation can happen during heated sample processing procedures including Polymerase Chain Reaction (PCR) amplification and DNA extraction. For this reason, it is essential to maintain ideal temperatures throughout these processes to avoid denaturing DNA and retain sample integrity [20].

**Enzymatic activity:** Enzymes involved in the breakdown of DNA are affected by temperature. Temperature-dependent activity is exhibited by nucleases, which are enzymes that catalyze the breakdown of phosphodiester links in DNA. Elevated temperatures have the potential to accelerate the enzymatic breakdown of DNA molecules by nucleases found in biological specimens. Thus, to reduce enzymatic DNA degradation and maintain sample quality, temperature management is crucial throughout sample processing and storage [21,22].



**Storage conditions:** The stability of DNA during sample storage is also influenced by temperature. Raising the temperature can hasten the deterioration of DNA, especially in biological samples that have been exposed to the environment for a long time. Therefore, it is crucial to maintain the proper temperature during the storage and transit of samples, such as by employing refrigeration or storing them at a low temperature [23].

**Forensic analysis:** Temperature-related changes in DNA stability can have an impact on forensic analysis since deteriorated DNA can produce inaccurate or partial profiles. Elevated temperatures at crime scenes or during sample processing may deteriorate DNA evidence, making it less suitable for forensic examination. Therefore, to preserve the integrity of DNA evidence and improve the precision of forensic investigations, forensic experts must take temperature control techniques into account when gathering, processing, and storing biological materials [24].

### Humidity

The environment's humidity levels can affect DNA stability in some ways. Elevated relative humidity fosters the growth of microbes, hence augmenting the likelihood of DNA contamination and nuclease destruction. Furthermore, environmental dampness can promote the hydrolysis of DNA, jeopardizing the integrity of DNA even further. Therefore, it is crucial to maintain the proper humidity levels during sample processing and storage to stop DNA deterioration and retain sample quality.

In biological samples like blood and saliva stains, humidity—the amount of water vapor in the air—is essential to the stability and integrity of DNA. The dependability and precision of forensic DNA analysis can be greatly impacted by the effects of humidity on DNA [25]. Forensic scientists must comprehend these impacts to create procedures that reduce DNA deterioration and maintain sample integrity. The following are the main ways that humidity influences the stability of DNA:

**Microbial growth:** In biological samples, high humidity levels foster the growth of microorganisms such as bacteria and fungi. Blood and saliva stains can become contaminated by microorganisms from the environment or human skin, which may cause DNA deterioration. The quality and integrity of the DNA sample are compromised by the introduction of nucleases and other enzymes brought about by microbial contamination, which can break down DNA molecules. Therefore, to reduce microbial contamination and maintain DNA integrity, humidity levels must be carefully controlled during sample processing and storage.

**DNA hydrolysis:** By promoting hydrolysis, the chemical process by which water molecules break down the phosphodiester links in DNA molecules, humidity affects

the stability of DNA. Elevated relative humidity raises the availability of water molecules, which encourages DNA hydrolysis and strand fragmentation. This process is especially noticeable in biological samples that have been left in humid conditions for a long time. Hydrolysis-induced DNA fragmentation can make forensic analysis more difficult by influencing the type and amount of DNA that can be used for profiling [26,27].

**Sample preservation:** The preservation of DNA in biological samples during storage and transportation might be impacted by ambient humidity levels. High humidity can encourage the formation of mold and mildew, which can deteriorate DNA and jeopardize the integrity of samples. Furthermore, atmospheric moisture can cause water droplets to develop on surfaces or containers holding samples, which may hydrolyze DNA and cause it to degrade. To protect DNA integrity during sample storage and transportation, it is crucial to maintain optimal humidity levels and use appropriate storage containers, such as airtight and moisture-resistant packing [28].

**Sample collection:** DNA stability can also be impacted by humidity conditions at crime scenes or when collecting samples. Environmental factors that can hasten the breakdown of DNA in biological samples left at the site include excessive humidity, exposure to rain, or dampness. When gathering biological samples, forensic investigators need to take humidity conditions into account to reduce DNA deterioration and maintain sample integrity for later analysis [29].

### Sunlight exposure

In biological stains, exposure to sunshine, especially Ultraviolet (UV) radiation, can cause DNA photodamage. DNA molecules degrade as a result of strand breakage and cross-linking brought on by UV light. Long-term sun exposure can seriously damage the DNA in blood and saliva stains, making forensic analysis more difficult. Thus, maintaining the integrity of DNA evidence during sample collection and storage requires limiting exposure to sunlight and using UV-protective techniques.

The stability and integrity of DNA in biological materials, such as blood and saliva stains, can be significantly impacted by sunlight exposure. Sunlight-induced Ultraviolet (UV) radiation, especially at UVB and UVC wavelengths, can cause a variety of DNA damage types, which in turn affects the precision and dependability of forensic DNA analysis. Forensic scientists must comprehend how sunlight affects DNA stability to create procedures that reduce DNA deterioration and maintain sample integrity [29]. The following are the main ways that exposure to sunshine alters DNA stability:

**UV-induced photodamage:** Both direct and indirect processes can cause DNA molecules to become photodamaged by UV radiation from sunshine. When UV photons are absorbed



by DNA molecules, they cause direct photodamage, which results in the creation of DNA lesions such as pyrimidine (6-4) pyrimidone photoproducts [(6-4)PPs] and Cyclobutane Pyrimidine Dimers (CPDs). Reactive Oxygen Species (ROS), which can oxidize and destroy DNA molecules, are produced in cells by UV radiation and cause indirect photodamage. These DNA lesions have the potential to compromise the integrity and stability of DNA strands by changing their structure and changing their chemical makeup [30].

**DNA strand breaks:** DNA strand breaks can occur when UV-induced photodamage cleaves the phosphodiester backbone of DNA molecules. Depending on the degree of UV-induced damage, both Single-Strand Breaks (SSBs) and Double-Strand Breaks (DSBs) may happen. Breaks in the DNA strand can cause DNA molecules to lose their integrity and obstruct the processes of transcription and replication. Furthermore, DNA strand breakage can compromise the quality and quantity of DNA available for profiling, which can result in the loss of genetic information and impact the validity of forensic DNA analysis [31].

**Cross-linking:** The creation of cross-links between neighboring DNA strands or between DNA and proteins can also be brought about by UV-induced photodamage. Reactive DNA lesions like CPDs and (6-4)PPs can covalently bond with nearby nucleotides or proteins to form cross-links. Cross-links can cause genomic instability and cellular dysfunction by obstructing DNA replication, transcription, and repair activities. Furthermore, the accuracy of DNA profiling may be compromised by cross-linked DNA molecules' resistance to forensic DNA analysis techniques such as DNA extraction and amplification [32].

**Sample preservation:** When biological samples are exposed to the outdoors or left at crime scenes, sunlight can hasten the deterioration of the DNA in such samples. Long-term sun exposure can cause significant DNA damage, such as strand breaks, fragmentation, and cross-linking, which compromises DNA samples used for forensic investigation. Thus, to maintain DNA integrity during sample collection and transit, it is crucial to reduce sunlight exposure and use the proper protective measures, such as covering samples with opaque materials or keeping them in UV-protected containers [33].

### Substrate type

The stability and preservation of DNA can be affected by the kind of substrate to which biological stains are applied. Fabric and paper are examples of porous substrates that may absorb and hold biological fluids, providing an environment that is ideal for the preservation of DNA. On the other hand, non-porous surfaces, such as metal and glass, might not be able to hold onto liquids well, which could result in fast evaporation and possible damage to DNA. To maximize DNA recovery and analysis, forensic techniques need to consider substrate properties [34,35].

### Oxygen exposure

The presence of oxygen can cause oxidative damage to DNA. Reduced DNA production and integrity can be the result of DNA strand breakage and changes brought on by exposure to high oxygen concentrations or reactive oxygen species. Oxygen participates in oxidative reactions that have the potential to harm biological components. In the presence of oxygen, Reactive Oxygen Species (ROS) such as free radicals can be produced. Degradation of DNA, proteins, lipids, and other biological components can result from these ROS reacting with and damaging these constituents. Lipid membrane deterioration may be caused by oxidative damage [36-38].

### Discussion

The concentration and integrity of DNA in blood and saliva stains are greatly impacted by environmental conditions, which also have an impact on the validity of forensic analysis. Comprehending these variables is crucial in formulating efficacious forensic procedures and guaranteeing the conservation of genetic evidence in criminal cases [39]. Forensic scientists can improve the precision and dependability of DNA analysis by regulating temperature, humidity, sunlight exposure, and substrate type, which will ultimately help in the administration of justice [40,41]. To sum up, the stability and integrity of DNA in blood and saliva stains are significantly influenced by environmental conditions, which in turn affects the accuracy and dependability of forensic DNA analysis. The main environmental elements that affect DNA stability and degradation include temperature, humidity, solar exposure, and type of substrate [42].

The stability of DNA is significantly impacted by temperature, which also affects enzyme activity and DNA denaturation [43]. High temperatures accelerate the destruction of DNA. To prevent DNA degradation and maintain sample integrity, temperature conditions must be carefully controlled both during sample handling and storage. Environmental humidity can affect DNA stability by facilitating microbial growth and DNA breakdown [44]. While moisture makes DNA hydrolysis easier and so leads to DNA fragmentation, high humidity encourages microbial contamination and enzymatic DNA destruction. It's essential to maintain the right humidity levels for sample processing and storage to reduce DNA deterioration and maintain sample quality [45].

DNA integrity in biological samples can be jeopardized by sunlight exposure due to strand breakage, photodamage, and cross-linking, among other DNA toxicity mechanisms. To maintain DNA integrity during sample collection and storage, it is imperative to minimize exposure to sunlight and implement protective measures [46,47]. DNA stability is also influenced by the kind of substrate used to deposit biological stains; porous surfaces hold fluids better than non-porous ones. To maximize DNA recovery and analysis, forensic techniques need to take substrate properties into account [48,49].



In conclusion, the stability and integrity of DNA in blood and saliva stains are greatly impacted by environmental conditions, which also have an impact on the precision and dependability of forensic DNA analysis [50]. To maintain sample integrity and reduce DNA degradation during the forensic process, forensic scientists must carefully take into account these elements and put the right procedures in place [51]. This will guarantee the correctness and dependability of forensic DNA analysis in criminal investigations. To solve these issues and enhance the field of forensic DNA analysis, further investigation and cooperation between forensic scientists, environmental researchers, and technological developers are necessary [52].

This study analyses the effects of common environmental factors on the ability of DNA to form an RFLP pattern. In this study, the dried blood stains were exposed to various environmental conditions such as Humidity, heat, and sunlight for 5 days. These blood stains were prepared from fresh unclotted whole blood collected in EDTA vacutainers and then 175 ul blood was transferred onto 100% cotton fabric and exposed to different conditions for 5 days. The samples were exposed to UV radiation by keeping them under BLE spectroline model XX-15 as a UV light source for 5 days. Similarly, the samples were exposed to heat, humidity, and soil for 5 days and then DNA was extracted using the organic extraction method. After this 1% of the total DNA was loaded onto 0.9% agarose gel and the RFLP pattern of these samples was obtained. The RFLP pattern slightly decreases with the increase in the exposure time. In the case of UV radiation, the RFLP pattern was not consistent. By this, it can be concluded that the UV radiation affects the molecular structure of DNA thus causing the poor RFLP pattern. The more DNA is degraded as the exposure time under UV radiation is increased [53-55].

### Significance

The comprehensive research on the impact of environmental conditions on DNA stability and degradation in biological stains, particularly blood and saliva stains, is extremely important in the field of forensic science. Here's a comprehensive comment explaining the relevance of this review study:

**Enhancing forensic DNA analysis:** The review paper explains how environmental variables such as temperature, humidity, sunlight exposure, and substrate type affect DNA stability. This understanding is critical for developing forensic DNA analysis techniques and processes, which will eventually improve the accuracy and reliability of DNA evidence in criminal investigations.

**Preservation of DNA evidence:** Understanding how environmental factors affect DNA concentration and integrity is critical for preserving DNA evidence. By understanding how temperature and humidity affect DNA degradation, forensic scientists may devise better preservation procedures to preserve the integrity of DNA samples throughout time.

**Optimizing forensic operations:** The study underlines the need of forensic scientists understanding how environmental variables influence DNA stability in order to enhance forensic operations. By controlling temperature, humidity, and other environmental conditions during sample collection, storage, and analysis, forensic professionals can reduce DNA deterioration and increase the integrity of DNA profiles used in criminal cases.

**Influence on criminal investigations:** The findings of this review research have a direct influence on criminal investigations since they emphasize the importance of environmental elements in DNA analysis. By taking these aspects into account, forensic specialists can improve the precision and reliability of DNA analysis, resulting in more accurate suspect identification, innocent people being exonerated, and effective case resolution.

**Advancing forensic science:** This review research helps to enhance forensic science by offering useful insights into the intricate interaction between environmental circumstances and DNA stability. By tackling the obstacles given by environmental conditions, forensic scientists may improve their procedures and maintain the integrity of DNA evidence, thereby benefiting the administration of justice.

### Conclusion

Environmental conditions have a substantial impact on the stability and integrity of DNA in blood and saliva stains, affecting the precision and reliability of forensic DNA analysis. Temperature, humidity, sunlight exposure, and substrate type are the primary environmental variables influencing DNA stability and degradation. High temperatures increase DNA degradation by processes such as oxidation and hydrolysis, resulting in DNA fragmentation. Maintaining correct temperature control during sample handling, storage, and transit is critical for preventing DNA degradation. Environmental humidity can stimulate microbial growth and DNA hydrolysis, resulting in DNA contamination and fragmentation. Controlling humidity levels is critical for maintaining DNA integrity throughout sample processing and storage.

Exposure to sunshine, particularly UV radiation, can induce DNA photodamage, resulting in strand breaks, cross-linking, and other types of DNA damage. Limiting sunlight exposure and implementing UV-protective techniques are critical for preserving DNA evidence integrity. The kind of substrate on which biological stains are produced can also influence DNA stability, with porous surfaces such as fabric maintaining DNA more effectively than non-porous surfaces. In conclusion, recognizing and managing these crucial environmental elements is critical for forensic scientists seeking to optimize DNA analysis techniques and assure the reliability of DNA evidence in criminal investigations.



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