

Review Article

Review of the Efficiency of Ten Different Commercial Kits for Extracting DNA from Soil Mixed Biological Samples

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Abstract

Soil-mixed bodily fluids are the most common kind of evidence at outdoor crime scenes. This biological evidence contains DNA, which is a key component of forensic science's ability to prove an accused person's guilt because it connects the victim and suspect to the crime scene and aids in identifying the offender and victim. The yield of DNA is significantly influenced by factors including temperature, humidity, storage environment, time since deposition, etc. DNA degradation is caused by a variety of microbes, bacteria, humic acid, and other substances present in soil. Nowadays for DNA extraction, a variety of commercial DNA extraction kits was used now. This paper's objective is to compare the efficiency of ten different commercial kits used to extract mixed DNA samples. It has been observed that samples stored at a low temperature (-20 °C) are the best for soil blood mixture samples. Compared to samples paired with other types of soil (silt, clay, and marshland), sand soil had the largest production of DNA using the QIAmp investigator kit (Qiagen). Blood Miniprep kit extractions were mostly inhibited, the control that amplified confirms that this kit was the worst in terms of DNA extraction potency. The samples with fewer dirt particles had a much greater yield of DNA.

Introduction

Outdoor Crime scenes frequently contain biological samples that have been buried in various types of soil. These materials are typically severely deteriorated, making analysis challenging. The presence of bacteria, temperature change, humidity, UV radiation, and other elements all have a role in the deterioration of biological material.

The autolysis of the cell membrane initiates the post-mortem destruction of the cell. The DNA is then released into the environment and, once in the soil, has three possible outcomes:

1. It can bind to minerals and humic substances like Humic Acid (HA).
2. It can be broken down by bacteria's DNases and used as a nutrient for the growth of plants and microorganisms.
3. It can be incorporated into the genome of the bacterium [1].

DNA's physical, chemical, and biological characteristics, as well as soil characteristics like pH, moisture content,

humic substance concentration, mineral content, and cation concentration, all affect how long-buried samples of DNA will remain viable. It also depends on DNA's interaction with specific minerals, humic substances, and organo-mineral complexes.

DNA analysis, also known as DNA testing or DNA profiling, is a technique used to study and compare DNA samples. It is widely used in forensic science, paternity testing, genealogy research, and medical diagnostics. DNA analysis can provide information about an individual's genetic makeup, relatedness to others, and susceptibility to certain diseases or conditions [2].

A type of environmental factor called soil has a number of elements that have the ability to destroy biological evidence, including UV radiation, microbes, enzymes, pH and chemical composition, moisture and temperature, and pH and chemical composition. There are 10 billion bacteria in one gram of soil, with hundreds of distinct species [3-4]. The identification of mixed biological soil evidence like blood, semen, saliva, urine, etc. can be greatly aided by soil analysis. Anywhere, whether it's open or closed, criminality can occur. Therefore, it is not a given that DNA evidence retrieved from a crime scene

More Information

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will always be of a high caliber and be present in adequate quantities.

The majority of sexual assault instances involve blood and sperm, which are ideal environments for germ growth. These microorganisms produce biochemicals that degrade blood's DNA. Blood DNA that has fallen to the ground and becomes mixed with soil is destroyed by soil microbes, rendering it unusable for examination. Extreme weather-exposed tissue samples quickly degrade DNA, rendering it unrecoverable. Living samples that contain DNA in the dirt are destroyed by the bacteria DNase as well as by humic and mineral compounds like Humic Acid (HA). Bacterial growth is accelerated by humidity, which also accelerates DNase. DNase activity increases with temperature, reducing DNA's half-life. UV exposure in particular can cause DNA cross-linking, DNA fragmentation, and DNA adduct formation [5].

Additionally, it may cause oxidation of lipids and denaturation of proteins. Maintaining the samples' evidential value for precise analysis and interpretation while minimizing deterioration is possible by following appropriate temperature, humidity, light protection, and contamination control methods. DNA is frequently unable to be created from the soil due to co-purified pollutants. DNA may now be swiftly, inexpensively, and extensively removed from many forms of soil with little washing required.

Extracting DNA from soil-blood mixed samples can be challenging due to the complexity of the sample and the presence of inhibitors. However, with appropriate modifications and techniques, it is possible to obtain DNA from such samples [6].

Collection of a representative soil-blood mixed sample using aseptic techniques. It is crucial to minimize external contamination during collection. Use a clean, sterile tool to scoop a portion of the mixture.

Removal of Debris and Cellular Material is begin by removing large debris and cellular material from the soil-blood mixture. Centrifuge the sample or use filtration methods to separate the solid fraction from the liquid fraction. Collect the liquid fraction, as it is more likely to contain DNA. To break open cells and release DNA from the sample, use a combination of physical and chemical methods. Mechanical disruption, such as bead beating or vortexing, helps break open cells and release DNA. Enzymatic digestion with proteases or lysozyme can also aid in cell lysis [7]. The goal is to disrupt cell membranes and release DNA into the solution. Purify the released DNA from the lysed sample. Silica-based column extraction kits, magnetic bead-based methods, or organic extraction using phenol-chloroform can be employed. These techniques aim to remove impurities, cellular debris, and inhibitors, allowing for the isolation of DNA. Assess the quantity and quality of the extracted DNA using spectrophotometry or fluorometric methods. This step helps determine the DNA concentration

and assesses its suitability for downstream applications. Ensure that the DNA is pure, free from contaminants, and of sufficient quality for further analysis [8].

The success of DNA extraction from soil-blood mixed samples can vary depending on factors such as the ratio of soil to blood, the condition of the sample, and the presence of inhibitors. It may require optimization and adaptation of the protocol based on your specific sample characteristics. Additionally, consulting relevant literature or seeking expert advice can provide valuable insights for DNA extraction from soil-blood mixed samples [9].

Environmental conditions can have various effects on soil that contains blood. These conditions can influence the stability of the blood components, the degradation of DNA, and the overall integrity of the sample.

Environmental factors affecting DNA yield

Environmental conditions can have various effects on soil that contains biological evidence. These conditions can influence the stability of these components, the degradation of DNA, and the overall integrity of the sample. Here are some environmental factors that can impact soil with blood [10-12].

- 1. Temperature:** High temperatures can accelerate the degradation of blood components, including DNA. Heat can lead to denaturation of proteins, enzymatic activity, and microbial growth, all of which can degrade DNA. Extreme cold temperatures may also impact DNA stability over prolonged periods.
- 2. Moisture:** Moisture levels in the soil can affect the preservation of blood components. Excessive moisture or waterlogging can promote microbial activity and enzymatic degradation, potentially leading to the breakdown of DNA. On the other hand, extremely dry conditions can also impact DNA stability, causing desiccation and damage to the sample.
- 3. Oxygen availability:** Oxygen levels in the soil can influence the preservation of blood components. In aerobic conditions (with sufficient oxygen), microbial degradation can occur more rapidly. In contrast, anaerobic environments (low oxygen) may slow down microbial degradation but can still affect DNA stability due to other processes.
- 4. pH:** The pH of the soil can affect the stability of blood components, including DNA. Extreme pH conditions, either highly acidic or alkaline, can denature proteins and degrade DNA. Optimal pH conditions for DNA preservation are typically around neutral pH.
- 5. Exposure to light:** Prolonged exposure to Ultraviolet (UV) radiation from sunlight can lead to the degradation of DNA. UV radiation causes the formation of thymine dimers, which can disrupt the DNA structure and affect its integrity.



It's important to consider these environmental factors when working with soil samples containing blood, especially if the goal is to extract DNA from the sample. Proper storage and handling techniques, including maintaining a suitable temperature, moisture, and light conditions, can help preserve the integrity of the blood components and DNA within the soil. Additionally, timely processing of samples and using appropriate DNA extraction methods can minimize the potential effects of environmental conditions on DNA quality and yield.

DNA extraction kits

DNA kits are a popular tool used for extracting and analyzing DNA samples. There are several types of DNA kits available, but the most common ones are designed for home use and typically include a set of materials and instructions for collecting and extracting DNA from various sources [13]. The exact contents of a DNA kit may vary depending on the manufacturer and the specific purpose of the kit, but here are some common components you might find in a DNA extraction kit:

- 1. Collection swabs or tubes:** These are used to collect DNA samples from the desired source, such as the inside of the cheek (buccal swabs) or other bodily fluids.
- 2. Preservation buffer:** This is a solution provided in the kit that helps stabilize and protect the DNA sample during transportation and storage.
- 3. Extraction reagents:** These are typically a set of chemicals and enzymes that are used to break down the cells and release the DNA. The specific reagents can vary depending on the kit and the type of sample being processed.
- 4. Tubes or plates:** These are containers provided in the kit for holding the DNA sample and reagents during the extraction process.
- 5. Centrifuge tubes:** In some cases, a DNA kit may include centrifuge tubes, which are used to separate the DNA from other cellular components during the extraction process.
- 6. Instructions manual:** A detailed guide or manual is typically included in the DNA kit, providing step-by-step instructions on how to collect the sample, extract the DNA, and prepare it for further analysis.

Advantages of using DNA commercial kits

DNA extraction commercial kits offer several advantages over traditional laboratory methods of DNA extraction. Here are some of the key advantages:

- 1. Convenience:** DNA extraction kits provide pre-packaged reagents and protocols, making the process

more convenient and time-saving. The kits typically include all the necessary components, such as buffers, enzymes, and purification columns, in optimized and ready-to-use formats. This eliminates the need for researchers to individually prepare and optimize reagents, saving valuable time and effort.

- 2. Consistency and reproducibility:** Commercial kits are designed to provide consistent and reproducible results across different samples and experiments. The protocols provided in the kits are optimized and standardized, ensuring that each extraction follows a consistent methodology. This helps to minimize variability between extractions and allows for better comparison of results between different studies or laboratories.
- 3. User-friendly:** DNA extraction kits are designed to be user-friendly, even for researchers with limited experience in molecular biology techniques. The protocols are often accompanied by detailed step-by-step instructions, which simplify the process and reduce the chances of errors. Additionally, most kits include user-friendly formats, such as spin columns or magnetic beads, which facilitate quick and easy purification of DNA.
- 4. Time efficiency:** The optimized protocols and pre-packaged reagents in commercial kits enable faster DNA extraction compared to traditional methods. The standardized procedures and optimized reagents reduce the number of steps and overall processing time required. This is particularly beneficial when working with large numbers of samples or when time is a critical factor in experiments or diagnostic workflows.
- 5. High DNA yield and purity:** DNA extraction kits are designed to maximize DNA yield and purity. The kits often include specific lysis buffers and enzymes that efficiently lyse cells and release DNA. The subsequent purification steps effectively remove contaminants such as proteins, RNA, and inhibitors, resulting in high-quality DNA suitable for downstream applications, such as PCR, sequencing, and genetic analysis.
- 6. Scalability:** Commercial DNA extraction kits are available in different formats and sizes, allowing for scalability based on experimental needs. Kits can be selected based on the sample type, starting material, and DNA yield requirements. Whether you need to extract DNA from a few samples or process high-throughput samples, there are kit options available to accommodate different scales of extraction.
- 7. Quality control:** Reputable commercial kits undergo rigorous quality control measures to ensure consistent performance and reliability. Manufacturers often



validate their kits through extensive testing, ensuring that the reagents and protocols deliver reliable and accurate results. This gives researchers confidence in the quality of the extracted DNA and the reproducibility of their experiments.

Disadvantages

DNA extraction commercial kits offer several advantages, but there are also a few potential disadvantages to consider:

- 1. Cost:** DNA extraction kits can be more expensive compared to traditional laboratory methods. The convenience and pre-packaged nature of the kits come at a higher cost, especially when processing a large number of samples. This can pose a financial challenge, particularly for researchers with limited budgets or those who frequently perform DNA extractions.
- 2. Limited customization:** Commercial kits provide standardized protocols and reagents, which may not be customizable for specific research requirements. If you have unique sample types, require modifications to the extraction process, or need to optimize the protocol for a specific downstream application, commercial kits may not offer the flexibility needed. In such cases, developing custom extraction methods may be necessary.
- 3. Potential for contamination:** Although commercial kits are designed to minimize contamination, there is still a risk of introducing external contaminants during the extraction process. Contamination can adversely affect downstream applications, leading to false results or unreliable data. Strict adherence to good laboratory practices and proper handling techniques can help mitigate this risk.
- 4. Compatibility issues:** While commercial DNA extraction kits are generally compatible with a wide range of sample types, they may not be suitable for all sample sources or conditions. Certain complex or challenging sample types, such as degraded DNA, Formalin-Fixed Paraffin-Embedded (FFPE) tissues, or samples with high levels of inhibitors, may require specialized extraction methods that are not readily available in commercial kits.
- 5. Limited control over reagents:** Using a commercial kit means relying on the pre-packaged reagents provided by the manufacturer. This limits the control researchers have over the quality and composition of the reagents used in the extraction process. In some cases, researchers may prefer to have more control over the sourcing and preparation of reagents to ensure the highest quality and consistency.
- 6. Dependency on manufacturer:** When using a

commercial kit, researchers rely on the manufacturer for the availability and consistency of the product. If the manufacturer discontinues the kit or changes the formulation or protocol, it can disrupt research workflows and require adaptation to new methods or re-optimization of protocols.

- 7. Despite these potential disadvantages, DNA extraction commercial kits remain widely used in laboratories due to their convenience, standardized protocols, and reliability. Researchers should carefully assess their specific needs, budget, and the compatibility of the kits with their samples and research goals before deciding to use a commercial kit or opt for alternative extraction methods.**

Materials and methods

This study is aimed at conducting both qualitative and quantitative analysis to determine the DNA yield from forensic mixed samples containing blood and soil, which have been subjected to various environmental conditions for a specific period. However, it is important to acknowledge a limitation of this study: as the storage time of the samples increases, their integrity is compromised, making it challenging to obtain accurate results.

In this paper, we analyze the impact of soil on the yield of DNA from mixed biological samples with the comparison of the efficiency of 10 different commercial DNA extraction kits from soil-mixed blood samples.

Results

After extraction of different types of samples (serial no. 8, Table 1), the Powersoill MoBio DNA extraction Kit gave successful results from the purification of DNA from soil samples for 24 hours at 4 degrees Celsius as an abundant amount of DNA was found with less contamination [3].

In Quantifier Trio Kit (serial no. 1, Table 1), Blood was mixed with soil at room temperature at 4 °C, and -20 degrees Celsius for 2 to 12 weeks. It was obtained that there were full STR profiles generated for room temperature and -20 °C stored sample whereas in the 4 °C stored sample full, partial, and null profiles were generated depending on the sample storage duration [1].

Quantifier Duo Quantification Kit (serial no. 3, Table 1) was used on the blood-stained cemented floor pieces, Black road concrete, and wall plaster which is then dried in an incubator at 40 degrees for 2 to 4 hours. The time duration for the same is 6 months to 3 years, thus which resulted in complete STR profiles [14].

DNA casework Kit (serial no. 4, Table 1) used 572 blood samples pipetted on cloth and plastic pieces Placed in the indoor (light and dark) and outdoor (light and dark) scenarios for 5 days to 12 months. Gave 65% profile obtained and 49%

of the complete profile was obtained from plastic whereas 51% was obtained from cloth samples [15].

In addition, Prepfiler forensic DNA extraction Kit and Promega DNA extraction Kit (serial no. 5 and 6, Table 1), were used when blood is mixed with soil at room temperature for 2 to 12 weeks giving the highest concentration of DNA [3].

SAPION Binding DNA extraction (serial no. 10, Table 1), gave the highest DNA profiles at 4 degrees for 24 hours which allows the complete saturation of the soil when blood is mixed with the soil [13].

In QIAmp investigator Kit(Qiagen) (serial no. 2, Table 1) used bloodstain made on 3 different fabrics such as jeans, cotton, and lycra which were then buried in 3 different types of soil that is, Sandy, marshy, and clay for 15 to 90 days which resulted in the best DNA profiles were obtained with samples buried during 15 and 30 days in sandy soil whereas in marshy soil DNA profiles were not able to obtain, as a result of the extremely degraded DNA because of the extending time and environmental conditions [16].

But Prepfiler TM forensic DNA extraction Kit (serial no. 9, Table 1) failed to extract PCR-ready DNA from soil contaminated with blood for 24 hours at 4 degrees Celsius and Blood Genomic DNA Miniprep Kit (serial no. 7, Table 1) gave the worst results when blood is mixed with soil for 2 to 12 weeks as it was not inhibited properly at room temperature [17].

Criteria and recommendations for efficiently extracting DNA from soil samples

The criteria to take into consideration for DNA extraction: Contamination must be carefully monitored to precisely get a representative DNA extract of the soil sample being analyzed in order to produce repeatable, educational, and trustworthy findings in soil forensic studies. Events at a crime scene that take place after a crime may bring contamination in addition to lab contamination as a consequence of soil transfer effects, thus it is important to take these into account. Ample sample size and numbers must be ensured during collection in order to accurately identify samples that do in fact come from a common source and to provide consistent and accurate results [18].

Sample contamination: Contamination of forensic biological samples is the unintended addition of foreign biological material to a sample, which may have an impact on the precision and dependability of forensic analysis. Contamination can happen at numerous points throughout the sample collection, handling, storage, or analysis. It can come from a variety of things, such as human mistakes, environmental variables, or defective equipment.

Contamination can have a substantial influence on how data are interpreted and may result in inaccurate conclusions or improper person identification. Any DNA analysis should be done with caution because of contamination, especially in forensic science [19-23].

Table 1: Extraction of blood mixed with soil samples using different types of extraction kits.

Serial no.	Types of kit	Types of sample	Storage condition	Time duration	Result	References
1	Quantifier trio kit	Blood mixed with soil	Room temperature, 4 °C, -20 °C	2 to 12 weeks	There were full STR profiles generated for room temperature and -20 °C stored sample, Whereas in the 4 °C stored sample full, partial, and null Profile generated depending on the sample storage duration.	[3]
2	QIAmp investigator kit (Qiagen)	Bloodstains were made on 3 different fabrics such as jeans, cotton, and lycra	Buried in 3 different types of soil. Sandy, marshy, and clay	15 to 90 days	The best DNA profiles were obtained with samples buried. During 15 and 30 days in sandy soil. Whereas in marshy soil DNA profiles were not able to be obtained, as a result of the extremely degraded DNA.	[1]
3	DNA IQ casework pro kit	572 blood samples pipetted on cloth and plastic pieces	Placed indoors (light and dark) and outdoor (light and dark) scenario	5 days to 12 months	65% of the profile obtained 49% of the complete profile was obtained from plastic whereas 51% was obtained from cloth samples.	[15]
4	Quantifiler® Duo Quantification kit	Blood was stained on cemented floor pieces, Black road concrete, and wall plaster.	Dried in an incubator at 40 degrees for 2 to 4 hours	6-month to 3 year	Complete, balanced STR profiles	[16]
5	Promega DNA IQ Extraction kit	Soil-blood mixed samples	Room temperature	2 to 12 weeks	Promega extracted samples completely.	[3]
6	PrepFiler Forensic DNA Extraction kit	Soil-blood mixed samples	Room temperature	2 to 12 weeks	PrepFiler extracted samples recorded the highest DNA concentrations	[3]
7	Blood Genomic DNA Miniprep kit	Soil-blood mixed samples	Room temperature	2 to 12 weeks	Blood Miniprep kit extractions were mostly inhibited, the control that amplified confirms that this kit was the worst in terms of DNA extraction potency	[3]
8	PowerSoil® MoBio DNA extraction kit	blood mixed with soil	24 hours at 4 °C to allow complete saturation of the soil.	24 hour	PowerSoil® MoBio DNA extraction kit is most successful in the purification of DNA from soil samples	[17]
9	PrepFiler™ Forensic DNA isolation kit	blood mixed with soil	24 hours at 4 °C to allow complete saturation of the soil.	24 hour	PrepFiler™ Forensic DNA isolation kit failed to extract PCR-ready DNA from soil contaminated with semen	[17]
10	SPION binding-based DNA extraction	blood mixed with soil	24 hours at 4 °C to allow complete saturation of the soil	24 hour	DNA extraction performed using the standard SPION binding protocol resulted in DNA Extraction	[18]



DNA contamination from outside sources can be introduced:

- 1) Before collection, combine the DNA with DNA from other sources.
- 2) During the process of gathering and keeping data, and/or
- 3) During laboratory testing.

Since dirt layers are frequently found on items like shoes, knives, and vehicle tires, layers should, wherever possible, be examined separately. According to soil characteristics and mineralogy as well as the form of contact, the principal impacts of soil transfer are particle size-selective to some extent. The tiny soil particles are usually lost first, leaving the coarse percentage of dirt that clings to objects available for study.

The fine soil fraction, which is made up of particles smaller than 150 m, will give the most accurate representation of the original soil since it doesn't include transfer effect artifacts. To perform an analysis utilizing just fine fractions for DNA profile comparisons, it may be advantageous to fractionate reference materials before extraction. It is standard practice to fractionate soil using sieve techniques. To get data with more accuracy and specificity about particle size distribution, Robertson, et al. advise using wet sieving rather than dry sieving. To avoid DNA contamination at this stage, the water and equipment used in this phase should be sterile (DNA free) [24-55].

Here are some common sources of contamination in forensic biological samples

1. **Cross-contamination:** This is the process by which biological material from one sample is transmitted to another sample, typically as a result of direct contact or improperly cleaned surfaces or equipment. Cross-contamination may occur, for instance, if a DNA analyst handles many samples while using the same gloves or tools without performing adequate cleaning.
2. **Environmental contamination:** Environmental pollutants including dust, airborne particles, or other biological elements present in the environment might affect biological samples. The integrity of the sample may be jeopardized by the introduction of certain contaminants during sample collection, packing, transportation, or storage.
3. **Laboratory contamination:** To prevent contamination, laboratories must uphold strict protocols and controls. However, contamination can occur as a result of human mistakes or poor sample processing. It may be anything as basic as not properly sterilizing the equipment or accidentally handling a sample while not wearing gloves.

4. **Sample collection contamination:** It's important to follow the right procedures and use the right equipment and containers while collecting biological samples. Failure to change gloves, the use of non-sterile collecting tools, improper sealing, and storage of the sample, or any of these can result in contamination.

Recommendations and precautions to reduce the risk of contamination

To minimize the risk of contamination and ensure accurate analysis, forensic laboratories employ various precautions and protocols: [12-14]

1. **Sterile techniques:** Using sterile gloves, disposable tools, and sterile containers during sample collection and analysis can minimize the risk of contamination.
2. **Separation and isolation:** Proper physical separation of samples and strict protocols for handling and storage help prevent cross-contamination between samples.
3. **Clean laboratory environment:** Forensic laboratories maintain clean and controlled environments to reduce the potential for environmental contamination. Regular cleaning and maintenance of equipment and surfaces are essential.
4. **Quality control measures:** Implementing quality control measures, such as regular validation of laboratory procedures and proficiency testing, helps identify and address potential sources of contamination.
5. **Chain of custody:** Maintaining a strict chain of custody, which documents the handling and storage of samples from collection to analysis, ensures accountability and helps identify any potential contamination points.

It is crucial for forensic scientists and technicians to be vigilant about contamination risks, follow established protocols, and undergo continuous training to maintain the integrity of forensic biological samples and the accuracy of their analyses.

Conclusion

Biological evidence at crime scenes can be compromised by environmental factors such as dirt, humidity, temperature, and UV radiation. DNA degradation is caused by bacteria, microbes, and humic acids in soil. DNA is crucial for convicting suspects, so ensuring its reliability is essential. Extracting biological evidence from mixed soil samples has been extensively investigated.

The Powersoill MoBio DNA extraction Kit, Quantiflier Trio Kit, Quantiflier Duo Quantification Kit, Promega DNA extraction Kit, and DNA casework Kit yielded the best results. Conversely, the Prepfilier™ forensic DNA extraction Kit and Blood Genomic DNA Miniprep Kit produced poor results when used with blood-mixed samples.



While extracting DNA from soil containing biological evidence can be challenging, various techniques can enhance the quantity and effectiveness of extraction. Commercial DNA extraction kits retrieve DNA from mixed biological evidence in soil. Designing the DNA extraction method should consider the specific soil-biological evidence combination. Testing different extraction buffers, sample ratios, incubation times, and temperature settings is necessary to optimize DNA yield and purity.

Proper sample preparation, including homogenization, sieving, and inhibitor removal, is crucial. Soil samples often contain PCR inhibitors that hinder further investigation. Taking extra steps during or after DNA extraction to eliminate or reduce these inhibitors is recommended. Enzymatic treatment or Solid-Phase Extraction (SPE) purification methods can help reduce or eliminate PCR inhibition.

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