جامعة نايف العربية للعلوم الأمنية
NAIF ARAB UNIVERSITY FOR SECURITY SCIENCES
تأسست ١٩٧٨ Est. 1978

Naif Arab University for Security Sciences
Arab Journal of Forensic Sciences and Forensic Medicine
المجلة العربية لعلوم الأدلة الجنائية والطب الشرعي
https://journals.nauss.edu.sa/index.php/AJFSFM

الجمعية العربية لعلوم الأدلة الجنائية والطب الشرعي
Arab Society for Forensic Sciences and Forensic Medicine

New Methodology for Non-destructive Identification of Body Fluid for Forensic Purposes

منهجية جديدة للتعرف على سوائل الجسم دون إتلافها واستخدامها كأدلة جنائية

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Received 19 Dec. 2023; Accepted 9 Apr. 2024; Available Online 11 Jul. 2023.

Abstract

The purpose of this research study was to test and subsequently validate a method based on the identification of biological fluids by nondestructive analysis, attributing phenotypic characteristics to biological stains. Specifically, a new methodology for non-destructive forensic histological analysis using Alternative Light Source 450 nm + orange filters was developed and validated - also testing its analytical efficiency and inherent degree of probabilistic error.

The validation process has been carried out through the creation of a standard database of known samples, in which were included blood-stains, semen stains, saliva stains and urine stains. Histological profiling with the observation of given parameters was included in the study such as fluorescence/absorbance, stain's shape, intensity of the signal, concentration of the signal etc.

160 reference samples were produced, which were used as a comparison method for subsequent histological profiling activities. Through a method validation procedure developed on four different tests of the technically investigated samples, specific useful ranges were identified for histological-forensic diagnosis of the biological stains, both known in the reference sample and unknown samples. Careful observation of the biological traces revealed several phenotypic characteristics, through which the study took shape. In conclusion, this experimental work made it possible to design and validate a

Keywords: Forensic sciences, forensic biology, identification, body fluids, validation study.

المستخلص

كان الغرض من هذه الدراسة البحثية هو اختبار والتحقق من صحة طريقة تعتمد على تحديد السوائل البيولوجية عن طريق التحليل غير المتلف لها، ونسب الخصائص المظهرية إلى البقع البيولوجية. على وجه التحديد، تم تطوير منهجية جديدة للتحليل النسيجي الجنائي غير المتلف باستخدام مصدر الضوء البديل 450 نانومتر + مرشحات برتقالية والتحقق من صحتها - كما تم اختبار كفاءتها التحليلية ودرجة الخطأ الاحتمالي المتأصلة.

وقد تم تنفيذ عملية التحقق من الصحة من خلال إنشاء قاعدة بيانات قياسية للعينات المعروفة، والتي شملت بقع الدم، وبقع السائل المنوي، وبقع اللعاب، وبقع البول. لقد تم إنشاء «التنميط النسيجي» مع ملاحظة المعلمات المحددة المدرجة في الدراسة (مثل: الفلوروسية/الامتصاص، وشكل البقع، وكثافة الإشارة، وتركيز الإشارة وما إلى ذلك).

تم إنشاء عينة مرجعية كقاعدة بيانات مفيدة للتوصيف النسيجي للعينات؛ باستخدام الضوابط الإيجابية والسلبية المناسبة، تم إنتاج 160 عينة مرجعية، ثم استخدمت كوسيلة للمقارنة لأنشطة التنميط النسيجي اللاحقة. من خلال إجراء التحقق من صحة الطريقة التي تم تطويرها على أربعة اختبارات مختلفة للعينات التي تم فحصها تقنيًا، تم تحديد نطاقات مفيدة محددة للتشخيص النسيجي الجنائي للبقع البيولوجية، المعروفة في العينة المرجعية وغير المعروفة (أي النتائج). وكشفت المراقبة الدقيقة للآثار البيولوجية عن العديد من الخصائص المظهرية، وهي إطار الدراسة. في الختام، مكّن هذا

الكلمات المفتاحية: علوم الأدلة الجنائية، علم الأحياء الجنائية، تعريف، سوائل الجسم، دراسة التحقق من الصحة.

Production and hosting by NAUSS

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doi: 10.26735/AXEM4254

new methodology for histological-forensic diagnosis through a non-destructive analytical methodology, which is particularly useful in the forensic field. Through this study is it possible to identify the histology of stains founded on the findings with a non-destructive analysis; furthermore it has to been specified that, according to the data obtained, it is possible to identify correctly the histological composition of the blood-stains but the histological identification of stains from semen, saliva and urine is still probabilistics.

العمل التجريبي من تصميم والتحقق من صحة منهجية جديدة للتشخيص الجنائي للنسيج من خلال منهجية تحليلية غير مدمرة، وهي مفيدة بشكل خاص في مجال الأدلة الجنائية. من خلال هذه الدراسة يمكن التعرف على أنسجة البقع المبنية على النتائج مع التحليل غير المدمر؛ علاوة على ذلك، يجب تحديد أنه وفقًا للبيانات التي تم الحصول عليها، من الممكن التعرف بشكل صحيح على التركيب النسيجي لبقع الدم، لكن التحديد النسيجي للبقع من السائل المنوي واللعاب والبول لا يزال احتماليًا.

1. Introduction

The purpose of forensic biological investigation, in relation to methodologies and technologies, is to identify elements of a biological nature that have validity from the investigation perspective; therefore, elements can give useful and indispensable information for the specific trace to an identified suspect [1]. The daily activities of forensic workers involve working on different types of biological materials and fluids such as: blood; seminal fluid; urine and saliva [2-4]. Therefore, a high efficiency in the identification of biological stains leads to greater efficiency in subsequent genetic type analysis [5].

In many forensic cases, findings offers limited amount of biological stains useful for the technical analysis. In these scenario, forensic scientists often haven't enough biological materials in order to perform both sierology analysis and DNA analysis. Nowdays, in this scenario, forensic operators normally have to choose which one analysis must to be perfomed (protocols prefers DNA analysis). Here the impact of this research study and of this new methods of analysis; in fact, in the cases with limited amount of biological materials for the forensic analysis, operators will be able to use a non-destructive methods for the sierology assessments and a destrucitve method – absolutely necessary in order to get the DNA results.

Currently, presumptive tests, such as the Kastle Meyer Test, and Rapid Stain Identification (RSID) confirmatory tests are used for histological-forensic investigations [6-7]. It should be noted that the aforementioned tests are based on a destructive analytical method, which causes several problems in the forensic context [8]. Therefore, the purpose of this research project is designed to precisely develop and validate; while also testing their analytical efficiency and inherent degree of probabilistic error, a new methodology for non-destructive histological-forensic analysis.

2. Materials and Methods

In order to have a useful database for the purposes of experimental activity and identify valid terms of comparison, five types of substrates were selected based on classical biological stains of forensic interest. These substrates were classified into porous and non-porous categories, while different chromes were selected for each of the aforementioned substrates.

The total number of substrates used for the construction of the reference sample (reference database) is 25 different substrates on which, then, different known amounts of biological stains and the negative and positive controls were added. The characteristics of each substrate used are listed below:

- **Wool:** porous substrate; 7 chromes (White, red, green, gray, yellow, blue, and black).
- **Cotton:** porous substrate; 7 chromes (White, red, green, gray, yellow, blue, and black).
- **Synthetic:** porous substrate; 7 chromes (White, red, green, gray, yellow, blue, and black).

- **Cardboard:** porous substrate; 2 chromes (White, brown)
- **Plastic:** non-porous substrate; 2 chromes (Black, white)

The biological matrices (stains) used in this project are urine, saliva, blood, and seminal fluid. A total of 1250 μL was used for each biological matrix, which was divided into 50 μL on the different substrates of the reference sample. Other accessory materials and instrumentation used for the experimental activity are the following items:

- P100 micropipette,
- Scissors,
- Stapler,
- I-Phone 13 Pro-Max camera,
- Alternative Light Source (ALS) with wavelength 450 nm,
- Orange filter,
- Distilled water; and
- PPE for dressing the operators.

In order to obtain standardized substrates that could be used in the creation of the biological stains database, rectangular surfaces having standardized dimensions of 3x4 cm were cut out; one (1) cm of the longest side of these rectangles, was allocated to nomenclature reporting information on the type of substrate, its color, and the biological matrix used. The result of this procedure resulted in square sub-areas of size 3x3 cm, reporting the biological stain that was the subject of the biological-forensic inspection.

For each individual substrate, two (2) rectangles were cut out for a total number of one hundred (100) samples of which twenty-five (25) were blood, twenty-five (25) were saliva, twenty-five (25) were urine, and twenty-five (25) were seminal fluid. A positive control and a negative control were always used in this experimental procedure.

After preparing the substrates, affixing corresponding labels for internal laboratory terminology, and loading all samples onto the substrates, the selected biological stains were incubated overnight at room temperature. The following day, the stage of trace inspection was initiated; this analytical procedure should always occur under dark conditions. The analytical-inspection activity is was conducted first, with the naked eye and, then, by the use of ALS 450nm with an orange filter (the aforementioned filter is applied to both the operator and the camera). Then, photo-documentation was conducted using the I-Phone 13 Pro-Max Camera. After observation, qualitative reports were made of the visibility or non-visibility of the track, based on the photographs taken. Preliminary selection of useful samples was made based on the possibility of identifying, or not identifying, the known and applied biological stain on the substrate. Samples that showed no visibility of the biological stain were excluded from the study; the others (80 samples out of 100) were then selected for subsequent experimental activities and data evaluation. The observation of these samples led to the assignment of various phenotypic characteristics, which were useful in the subsequent identification of the histological nature of biological fluid.

The following characteristics were observed in this study:

- **Absorbance:** Intensity of electromagnetic radiation that is absorbed by a body,
- **Fluorescence:** Property whereby some substances, when investigated by incident electromagnetic radiation, re-emit with a very short delay, radiation of a different frequency from the incident and substance-dependent frequency,
- **Intensity:** Describes the bright of a light source,
- **Concentration:** Affluence or convergence of considerable magnitude at a given point, and
- **Edge:** Contour of a figure.

The profiling of all the biological stains in the database led to the probabilistic calculation of a series of profiles, calculated according to the five aforementioned characteristics, according to which one is likely to discriminate the nature of the different biological stains used for the study. Once the database profiling was finished, a validation study was conducted on the efficiency and reliability of the method starting with unknown samples to this investigator.

3. Results and Discussions

The observed samples were subjected to various tests. A preliminary selection was made of the substrates where the stains were visible and consequently identified as samples useful for the study. The phenotypic characteristics listed above were profiled based on absorbance, fluorescence, intensity, and edge of the selected samples. This step was referred to as the "1st Level Test" and included all samples on which incubated biological stains were visible by the above-mentioned inspection methodologies.

The samples that proved useful, and were included in the 1st level test, are:

- Wool (white, green, yellow, gray, black, red),
- Cotton (white, green, yellow, gray),
- Synthetic (white, green, yellow),
- Carton (white, brown), and
- Plastic (white, black).

With the abovementioned samples, a reference was created; according to the obtained data, it has been estimeed a “profile” for each body fluid – with specific indication of the probability of presence. This reference sample will be used as a benchmark for testing the efficiency and reability of this new methods of forensic analysis.

In the next step, identified as a "2nd level test" was conducted using all reprofiled samples. This procedure was conducted to verify the reliability of the method and to ensure that the samples were not known to the “profiling operator”, each sample was assigned an identification number by an operator outside of the test. The results obtained in the 1st and 2nd level tests were represented with 6 six graphs, including one for each stain and two for highlighting the total performance.

- Performance was evaluated by an attribution probability classified into the following categories:
- **Certain:** when the calculations achieve 100% attribution probability,
- **High:** when calculations-including between probabilistic values-lead to identification of the stain with the highest value,
- **Intermediate:** when the calculations-including between probabilistic values-lead to identifying the stain with the intermediate value, and
- **Low:** when calculations-including between probabilistic values-lead to identifying the stain with the lesser value.

The study was extended to two additional tests:

The "3rd level test" was aimed to establish profiling as objective. A total of 80 samples were assigned identification number to neutralize their identity, then make, were given a code to further modify samples, so the nature of the biological fluid would not be known a priori.

A total of 80 samples were individually profiled by three operators. The profiles were then compared and averaged to obtain a common profile (comprising the individual ratings of the three operators). After the operators' comparison, some samples were excluded from the study since the amount of the biological fluid present on the substrate was not significant enough and sufficient to profile.

With these selected samples, a "4th level test" or a repetition of the previous test was carried out, the purpose of was to counter-verify and confirm the profiling data obtained from the individual operators and their overall averages. Below are graphs of the performance of individual stains and totals.

The performance of blood has not changed at all. Probabilistic attribution is certain at 100%.

The performance of saliva is improved. Among the selected samples, all are at high probability attribution, only one has a low probability of attribution.

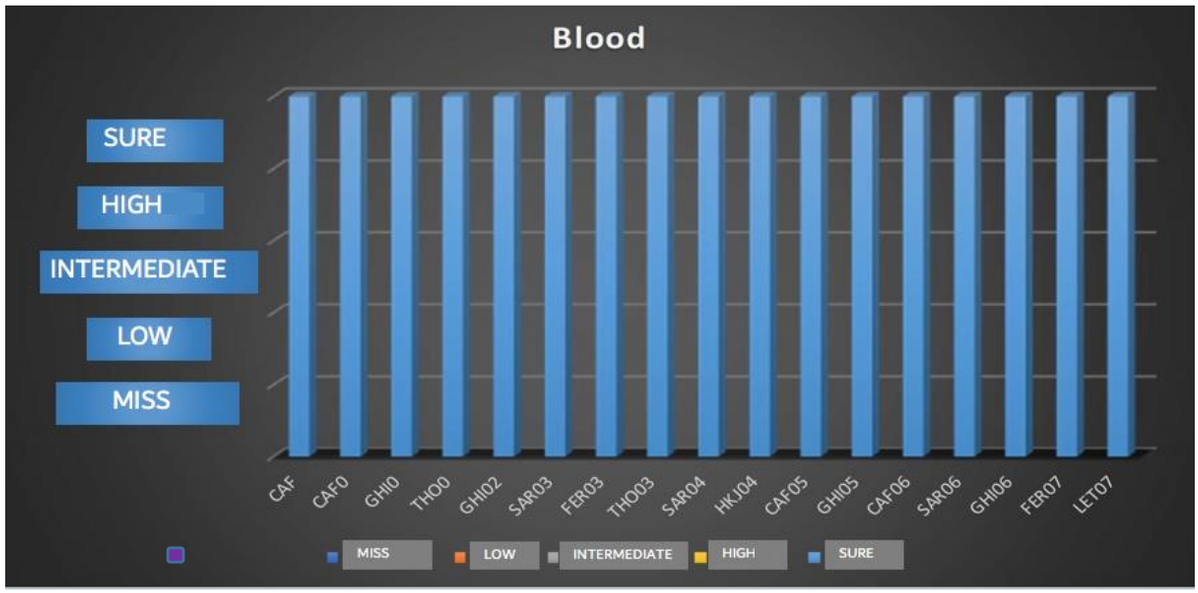

Figure 1- *Performance in the identification of the blood stains.*

Figure 2- *Performance in the identification of the saliva stains.*

The performance of semen improved from the previous one, with only two average attribution probabilities compared to the three average attribution probabilities obtained in the previous tests.

The performance of urine is slightly lower than the previous data because there are only intermediate probabilistic attributions.

In general, the use of this methodology had maintained improvements in probability that netted 45% certain probability, 26% high probability, 24% intermediate probability, only 5% low probability of attribution, and 0% missed probability. The study confirmed that Blood had 100% analytical efficiency. In 71% of the samples, the probability of attribution

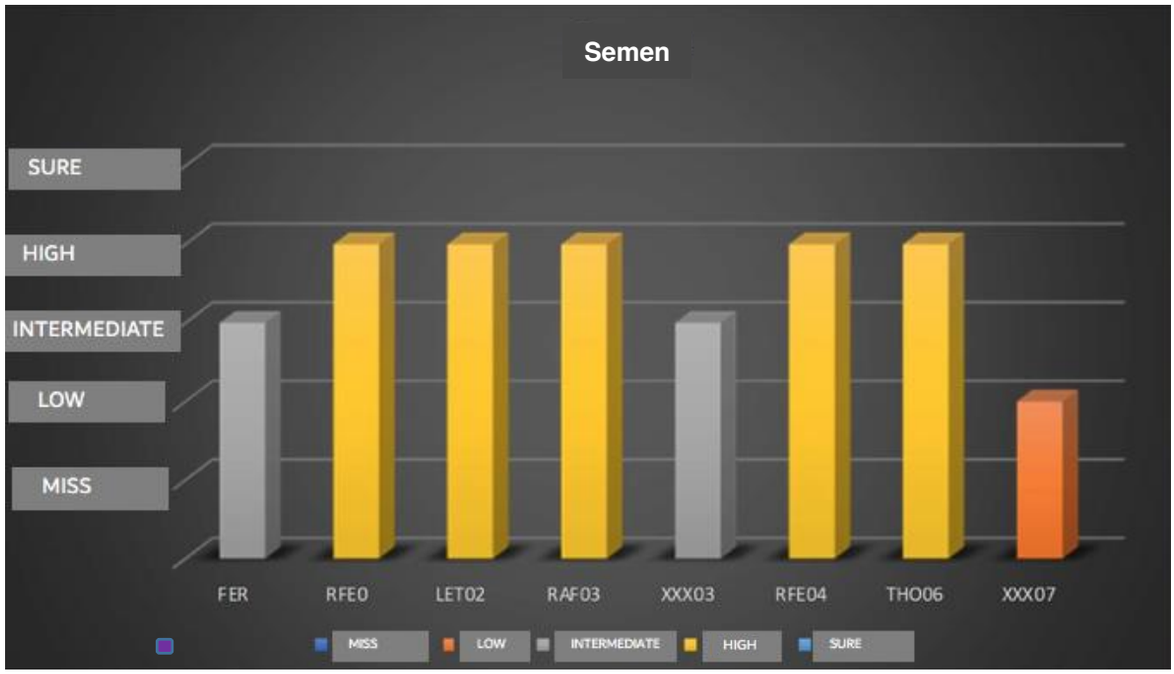

Figure 3- *Performance in the identification of the semen stains.*

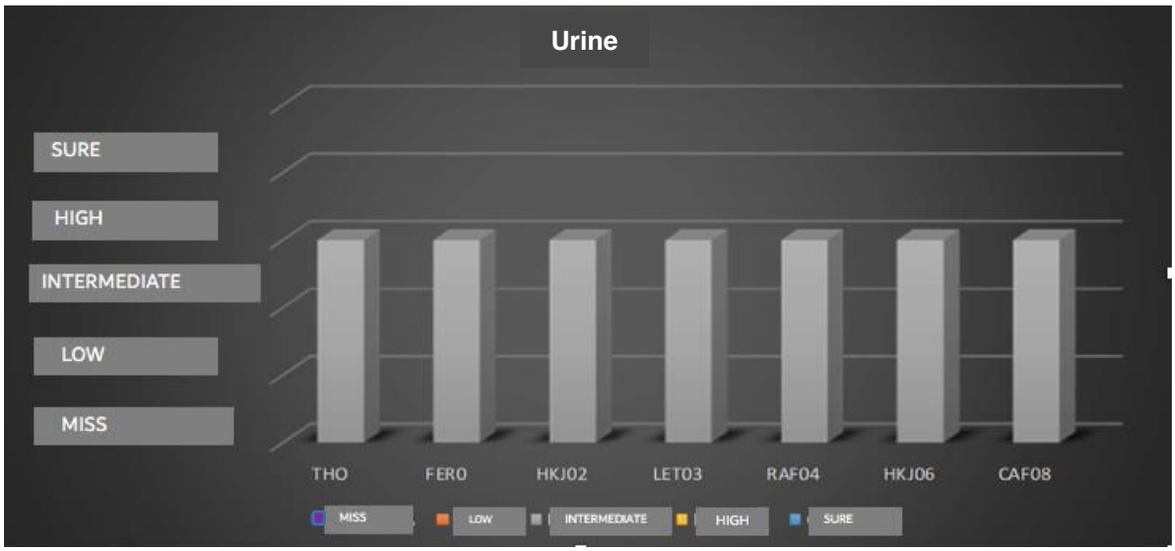

Figure 4- *Performance in the identification of the urine stains.*

ranged from certain to high with a non-destructive method, while in only 5% of cases, the probability of attribution was low. Urine was rated as the most complex biological fluid and the totality of attributions was in the intermediate range.

Figure 5- *General performance in the stains identification.*

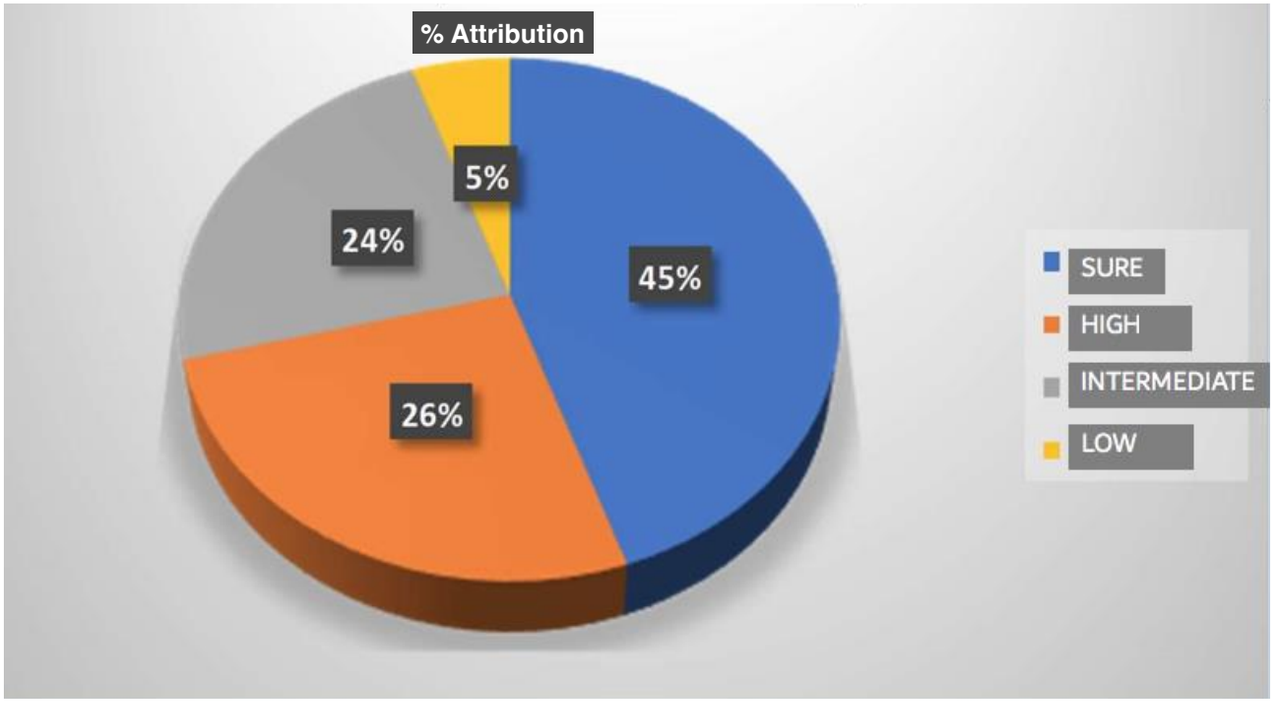

Figure 6- *A graphic illustration of the percentage of the range of attribution.*

4. Conclusion

This study had successfully produced a new methodology for non-destructive forensic histological analysis, using Alternative Light Source 450 nm + orange filters. This procedure was developed, validated, and tested the analytical efficiency and inherent degree of probabilistic error. The validation method procedure involved four different tests of the technically investigated samples for histological-forensic diagnosis of the biological stains, both known in the reference sample and unknown findings. Careful observation of the biological traces revealed several phenotypic characteristics.

Due to a combination of data from different selected parameters – such as fluorescence/absorbance, stain's shape, intensity of the signal and concentration of the signal – it is possible to obtain a new method for the histological identification of the biological stains detected on the findings. Furthermore, it is very important to highlight that this new method is a non-destructive procedure, so it will possible to perform both the sierology analysis both the DNA analysis in cases in which the biological stains are very limited in terms of quantity.

In conclusion, this experimental work made it possible to design and validate a new methodology for histological-forensic diagnosis through the use of a non-destructive analytical methodology, which appeared as useful advancement in the forensic field.

Conflict of interest

The authors declare no conflicts of interest.

Source of funding

The authors received no financial support for the research, authorship or publication of this paper.

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