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Effectiveness of the Jaffe Test for Urine Mark on Denim Fabric Over a 15-Day Period

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Abstract: ***This research has evaluation of forensic analysis of urine stain on denim fabric. Application of Jaffe test for the creatinine measurement for 15 days duration. Urine stains can be useful evidence for criminal investigations. Denim is commonly worn clothing and gives rise to interesting forensic opportunities. The Jaffe test confirms urine stains presence in an object. It is a colorimetric technique that ascertains creatinine levels in an alkaline solution. The Jaffe test uses interaction between picric acid and creatinine to yield this result. The steps involved in Jaffe's test is described. Involves sampling, preparation, spectrophotometrically analysis, etc. To allow interference from the various compounds either in the urine or in the fabric, a protein-free filtrate is needed. Furthermore, urine detection is more reliable when using direct on denim testing than swabbing. Analysis of urine stains on denim come with many difficulties. The construction of denim itself has been investigated as a likely source of interference. The essay discusses the wider implication of urine detection in forensic cases. Detecting urine could help establish a timeline of events and can corroborate witness statements over that time period. In conclusion, the use of analytical tests like the Jaffe test with forensic techniques may help in the ability of urine analysis done over 15 days on denim fabric to be corroborated and validated. This approach leads to a thorough and comprehensive analysis. This will help in analysing evidence. Furthermore, it will also help in implied legal consequences. Most importantly, it strengthens the integration of the judicial process.***

Keyword: Presumptive test, Urine stain, Jaffe test, Creatinine, Picric acid, False positive.

I. INTRODUCTION

In forensic science, identification of different bodily fluids and analysing them is quite an effective tool in the investigation of crimes. Most especially, in the case of sexual assault, murder, and other violent crimes. Urine is one of the traces at crime scenes. Urine can help establish the timeline of events at a crime and witness corroboration. It can be important to connect a suspect to the crime. Forensic specialists must effectively recognize urine as it is an essential piece of information that can affect the outcome and course of an investigation. Presumptive Urine Testing has increasingly become an important first step in confirming that urine is present. The Jaffe test is one of the most commonly used methods to detect becomes a complex compound called creatinine, which is found in urine, when medicated by a sodium picrate in an alkaline medium, solution. The urine can be identified through the characteristic colour change involving the reagent and the substrate. This speed evaluation helps forensic professionals decide their further interrogative actions.

Identifying a person through urine testing is not the only feature. It also informs the conditions under which a crime occurred. For example, in a sexual assault case, urine identification can help identify whether the victim was attacked at that spot or the suspect was at that place. In addition, tests on urine can aid in investigations related to drugs by discovering substances that could point towards the usage or intoxication by drugs at the time of the incident. Since forensic investigation is highly essential, the preliminary test such as the Jaffe test provides a quick result for further investigation. In time-sensitive situations where you have no time to waste, the ability to get instant results is invaluable for collecting and analysing evidence.

Urine stains at a crime scene can also change as time goes on. As time passes, numerous chemical and physical changes occur that can influence both the composition of urine stains and the reliability of presumptive tests, such as the Jaffe test. Studies have shown that environmental conditions that include heat and light can begin to break down urine stains almost instantaneously after the urine is laid down. This breakdown of the stain may change the stain's chemical properties which may interfere with presumptive tests and yield negative results. It's important for forensic professionals to understand how urine stains alter in terms of age. Through the alteration of urine stains, the investigation approach and the outcome may vary.

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The present report shall examine the modalities and implications of presumptive urine testing from a forensic perspective, mainly focusing on the Jaffe test and its efficacy over time. If professionals are aware of these issues and their limitations, they can use them to carry out investigations effectively. This study proposes best practices for urine testing in forensic science to enhance overall efficiency in an investigation and effectively serve justice.

II. URINE

Urine is a liquid waste product that is produced by the body's kidneys and use in excreting the waste. The main constituents of urine are water, salts and nitrogenous wastes which are used to excrete excesses and maintain homeostasis. Urine is formed via Glomerular filtration, reabsorption, and secretion processes. Healthy individuals also excrete a small amount of protein. Urine has a characteristics yellow color. After urine has left the body, it can pick up an odour due to the ammonia that is released when bacteria break down urea.

A. Urine Composition

Urine is made up of 95 percent water and various ions and small organic molecules. Urine composition is necessary to study kidney function and any disease occurring thereof. Urine is comprised of a variety of components, and below is the brief description of its key components and their function.

1) Water: Water is the main constituent of urine as it constitutes 91% to 96% of the total urine. The percentage varies depending on how hydrated you are. Thus, if your urine is concentrated you are dehydrated. If your urine is diluted you are more hydrated.
2) Urea: Urea is formed from liver proteins' breakdown. It is a nitrogenous waste matter. It is about 2% of the composition of urine (9.3 g/L). Urea is non-toxic and the main way the body removes excess nitrogen.
3) Chloride: Chloride ions are important electrolytes that are present in urine to the tune of approximately 0.6 % (i.e. approximately 1.87 g/L). Chloride helps in osmotic balance in the body through liquid regulation in the body.
4) Potassium: Urine contains another electrolyte, potassium, estimated at 0.6% (about 0.750 g/L) of its content. Calcium for the body is essential as it transmits nerve impulses and muscle contractions.
5) Sodium: Nearly all 17.68g (or 5.5%) excreted in urine daily. Sodium, like chloride and potassium, is important for fluid balance and cellular functioning.
6) Creatinine: Muscle tissue breaks down to produce creatinine. It makes up about 0.1 % of the urine (0.670 g/L). It is made at a fairly constant rate, and doctors monitor how much a patient has in their blood to assess kidney function.

B. Formation Of Urine

1) Glomerular Filtration: The functional unit of the kidney is the nephron; the site of first formation of urine. Blood comes into the nephron from the afferent arteriole into the glomerulus, a capillary network. In this process, blood is filtered with a semi-permeable membrane which allows small particles such as water, ions and small molecules to pass through but retains larger particles such as proteins and blood c ells. The hydrostatic pressure process which causes glomerular filtrate formation contains water, electrolytes, glucose, amino acids, urea, creatinine and other low molecular size solutes.
2) Reabsorption: Following filtration, the next stage involves reabsorption. As the filtrate travels through various parts of the nephron, including the proximal convoluted tubule, loop of Henle, distal convoluted tubule, and collecting duct, reabsorption of useful substances takes place. About 99% of the water is reabsorbed along with numerous solutes like glucose, sodium ions and bicarbonate. This selective reabsorption is essential for electrolyte balance and fluid homeostasis in one's body.
3) Secretion: The last step in urine formation is secretion. During this step, additional wastes and excess ions are actively transported from the blood into the tubular fluid. The distal convoluted tubule and collecting duct mainly undergo this process. Hydrogen ions, potassium ions, creatinine, urea and drugs are excreted from the blood into filtrate to be excreted as urine. This process is helpful for the management of acid-base balance and the removal of toxins.

III. JAFFE'S TEST

The Jaffe test is a popular chemical test that detects creatinine, which is an important urinary metabolite. The test principle states that the creatinine in alkali solution reacts with picric acid to give an orange red color. You can measure how intense a color is either with the naked eye or with instruments. Either way, it's a good judge. Therefore, it's a simple yet reliable way to measure creatinine levels. The test was developed by Max Jaffe in 1886. After this, the test found extensive applications in clinical biochemistry. This was to monitor the function of the kidney.

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Another application was for forensic science to identify urine. This is because creatinine is relatively stable under various conditions. It is one of the simplest tests which gave results quickly and which is reproducible in the laboratory.

IV. MATERIAL AND METHODS

1) Sample Material:
- Denim fabric was chosen as the substrate for this test due to its application in clothing.
- The fabric was cut into equal-size pieces evenly. Therefore, it can be used for testing properly. Collected a fresh human urine sample from a healthy volunteer under hygienic conditions.

2) Chemical reagent:
- The chemical reagent is the saturated aqueous solution of picric acid and sodium hydroxide (NaOH) whose concentration is 1N and uses as the alkaline medium and distilled water.

3) Instrumentation and Apparatus:
- Beaker - Used for preparation and mixing of solutions.
- Test tube - For small scale reactions and preparing reagents.
- Test Tube Stand - To hold test tube while doing reactions.
- Cotton swab - For collection of Urine residue from Denim Sample.
- Measuring Cylinder - For measuring liquids to accurate volume.
- Dropper - For adding reagent drop-wise.

4) Sample preparation:
- A fresh urine sample was obtained from a healthy volunteer which was applied uniformly on 15 pre-cut denim pieces. The stained pieces of fabrics were dried at room temperature and taken outdoors for conditioning in the natural environment. The remedying conditions relied on temperature, humidity and sunlight. The installation was designed to replicate the urine stains that we find in daily life. The Jaffe test was utilized to evaluate denim samples at increments beginning with day 0 and ending with day 15. The environmental influence on urine stain detection over time on denim could thus be measured.

5) Preparation of Reagent (Jaffe's test):
- Prepare a 1 N NaOH (sodium hydroxide) in a 25 ml of distilled water:
  - Normality: Normality is a concentration term, i.e. it is defined as the number of equivalent solute per liter of solution.
  - Formula for Normality Calculation,

$$N = \frac{Weight\ of\ solute\ (g)}{Equivalent\ weight\ of\ solute\ (g)} * \frac{1}{Volume\ of\ solution\ (l)}$$

  - Rearranging to find the weight of solute:

Weight of solute(g)= N * Equivalent Weight (g)*Volume of solution (L).

- Procedure:
  - ✓ Determine the known values:
    - N=1 N (target normality)
    - The equivalent weight of NaOH is 40.00 g/mol as it gives 1 OH- ion on the release of 1 mole.
    - The total amount of solution, 25 ml or 0.025 L.
  - ✓ Substitute into the formula:
    - weight of solute (g) = 1 * 40.00 * 0.025 Weight of solute (g)

= 1.00 g
  - ✓ weight the NaOH:
    - weigh exactly 1.00 g of NaOH by using an analytical balance.
  - ✓ prepare the solution:
    - dissolve the weighed NaOH in about 20 mL of distilled water in a beaker.
  - ✓ Mix thoroughly:
    - Ensure the solution is well mixed by inverting or stirring.
  - ✓ Picric Acid Solution:
    - Use a cylindrical glass to take your picric acid to prepare reagent solution.

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✓ Making a Reagent Solution:
- In a clean test tube, take 1 ml of prepared 1N sodium hydroxide solution.
- Now add 1 mL picric acid solution to the same test tube.
- Mix the reagent gently but sufficiently to ensure it gets mixed with reagent. If available, swirl the test tube with a vortex mixer that will facilitate mixing.

V. ANALYSIS OF THE JAFFES TEST FOR URINE MARK ON DENIM FABRIC OVER A 15 DAYS PERIOD:

To detect creatinine in urine stains, perform a Jaffe test and follow the steps.

1) Prepare the Cotton Swab:
- Soak a cotton swab in distilled water. This will help in collecting the urine sample effectively.

2) Collect the Urine Sample:
- Use the damped cotton swab to take a sample from the cloth piece having urine stain. Lightly scrub the swab against the mark so that you collect any transference present.

3) Add Reagent Solution:
- Once 1 mL of the standard reagent solution (consisting 1ml of picric acid with 1ml of 1N sodium hydroxide) is added to the cotton swab taken after collection of samples, it is added.
- This substance will undergo a chemical reaction with urine creatinine.

4) Mix and Observe:
- Blend the chemical agent well on the cotton swab with the sample. Wait for a few minutes to see any changes in color.
- This is a positive creatinine test which shows the presence of urine in the sample according to distinct color change (normally orange-red color).

Day 1
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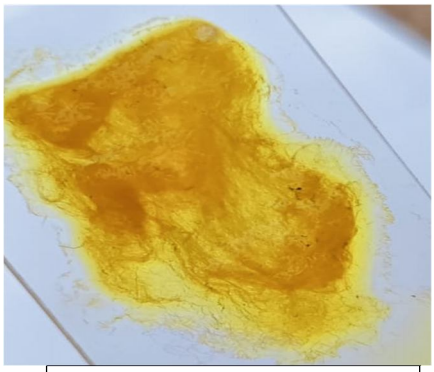

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Day 3
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Day 4
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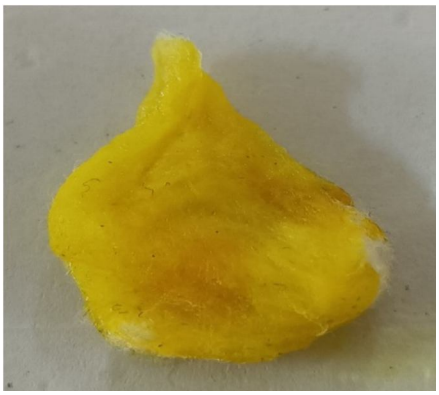

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Day 6
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Day 7

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Day 8

29/Nov/2024, 11:10 PM

Day 9

30/Nov/2024, 03:10 PM

Day 10

01/Dec/2024, 04:10 PM

Day 11

02/Dec/2024, 02:20 PM

Day 12

03/Dec/2024, 04:25 PM

Day 13

04/Dec/2024, 02:15 PM

Day 14

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Day 15

06/Dec/2024, 03:45 PM

Fig.(d): ANALYSIS OF JAFFE TEST OF URINE WITH THE PASSAGE OF TIME

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❖ OBSERVATION TABLE:

DAY	OBSERVATION
Day 1	Slightly orange color
Day 2	Slightly orange color
Day 3	Less color change compared to previous day
Day 4	Clear, slightly orange color
Day 5	Less color change
Day 6	Dark orange-red color
Day 7	Clear, light orange color
Day 8	Clear, light orange color
Day 9	Dark orange-red color
Day 10	Slightly orange color
Day 11	Slightly orange color
Day 12	Clear orange color
Day 13	Clear orange color
Day 14	Clear orange color
Day 15	Dark orange color

VI. RESULT AND DISCUSSION

A. Results

- During the Jaffe test which lasted for 15 days, it was noted that all the denim samples produced orange coloration which indicates creatinine presence.
- The Colour Intensity kept changing with days.
  - Days 1, 2, 4, 7, 8, 10, 11 and 12 had a light to clear orange colour.
  - There was a slight decline in colour on days 3 and 5.
  - Days 6, 9, 13, 14, and 15 were painted in darker orange and orange-red.
- 89The Jaffe reaction is a reliable test for detecting urine stains in denim fabrics. Creatinine showed stable results at the time points up to two weeks under environmental conditions.

B. Discussion

- The consistent presence of creatinine and colour alteration suggest that urine stains on denim are reliably detectable up to 15 days using the Jaffe test.
- The changing colours may also be caused by factors in the environment like temperature, humidity, sun-warmth, and urine composition.
- The denim is absorbent in nature which may help it retain the components of urine and other stains for a longer time compared to other fabrics.
- The Jaffe test is a reliable and economic method in forensic investigations for the detection of drugs of abuse in biological samples.
- In the future, more specific reagents, linking with DNA analysis, portable field-deployable devices may come to use for urine stain detection in forensic sciences.
- Analysing samples from the crime scene properly gives the information and most likely remain unchanged.
- These findings justify the use of presumptive tests like Jaffe test and advanced confirmatory methods to arrive at a proper conclusion at a forensic purpose.

VII. CONCLUSION

The method used in this study of presumptive testing of urine stains on denim fabric for 15 days using the Jaffe test has been effective and can be used for reliable detection of creatinine. The positive results shown throughout the study confirm that the Jaffe

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test is reliable enough for forensic investigation in identifying urine helpful for linking a suspect to the scene of a crime and linking to others.

Urine might be located on a cloth despite it being there for some time now. This is why urine analysis is important. Although there were differences in colour intensity of the samples because of environmental factors, the Jaffe test is effective. It shows that forensic people should know the biochemistry of urine and all factors that may interfere with its detection.

In the end, forensic scientists should priority urine analysis as important evidence. Types of evidence like the Jaffe test help give an endorsement that can impact case results. It contributes to social justice. The development of forensic techniques and further research on biological evidence will reinforce urine analysis as an important component of forensic investigations.

REFERENCES

[1] Libretexts. (2024b, October 5). 24.3A: Overview of Urine Formation. Medicine LibreTexts. https://med.libretexts.org/Bookshelves/Anatomy_and_Physiology/Anatomy_and_Physiology_(Boundless)/24:__Urinary_System/24.3:_Physiology_of_the_Kidneys/24.3A:_Overview_of_Urine_Formation.

[2] Delanghe, J. R., & Speeckaert, M. M. (2011). Creatinine determination according to Jaffe--what does it stand for? Clinical Kidney Journal, 4(2), 83–86. https://doi.org/10.1093/ndtplus/sfq211

[3] Measurement of creatinine by Jaffe's reaction--determination of concentration of sodium hydroxide required for maximum color development in standard, urine and protein free filtrate of serum. (2002, March 1). PubMed. https://pubmed.ncbi.nlm.nih.gov/12635710/

[4] Yasmin, S., Kumar, A., & Bora, D. (2024). Comprehensive study of various chemical samples in forensic toxicology: Focus on urine sample. In Annex Publishers, Journal of Forensic Science & Criminology (Vol. 12, Issue 1, p. 103) [Journal Article]. https://www.annexpublishers.com/articles/JFSC/12103-Comprehensive- Study-of-Various.pdf

[5] Laboratory orientation and testing of body fluids and tissues for forensic analysts | Home | National Institute of Justice. (n.d.). National Institute of Justice. https://nij.ojp.gov/nij-hosted-online-training-courses/laboratory- orientation-and-testing-body-fluids-and-tissues/home

[6] How do I prepare 1N NaOH in 100ml water? - ECHEMI.com. (n.d.). ECHEMI https://www.echemi.com/community/how-do-i-prepare-1n-naoh- in-100ml-water_mjart22040910743_135.html

[7] Dolscheid-Pommerich, R. C., Klarmann-Schulz, U., Conrad, R., Stoffel- Wagner, B., & Zur, B. (2016). Evaluation of the appropriate time period between sampling and analyzing for automated urinalysis. Biochemia Medica, 82–89. https://doi.org/10.11613/bm.2016.008

[8] Stability of common analytes in urine refrigerated for 24 h before automated analysis by test strips. (2000, September 1). PubMed. https://pubmed.ncbi.nlm.nih.gov/10973869/

[9] Nisbet, L. A., DiEmma, G. E., & Scott, K. S. (2023). Drug stability in forensic toxicology. Wiley Interdisciplinary Reviews Forensic Science, 5(4). https://doi.org/10.1002/wfs2.1481

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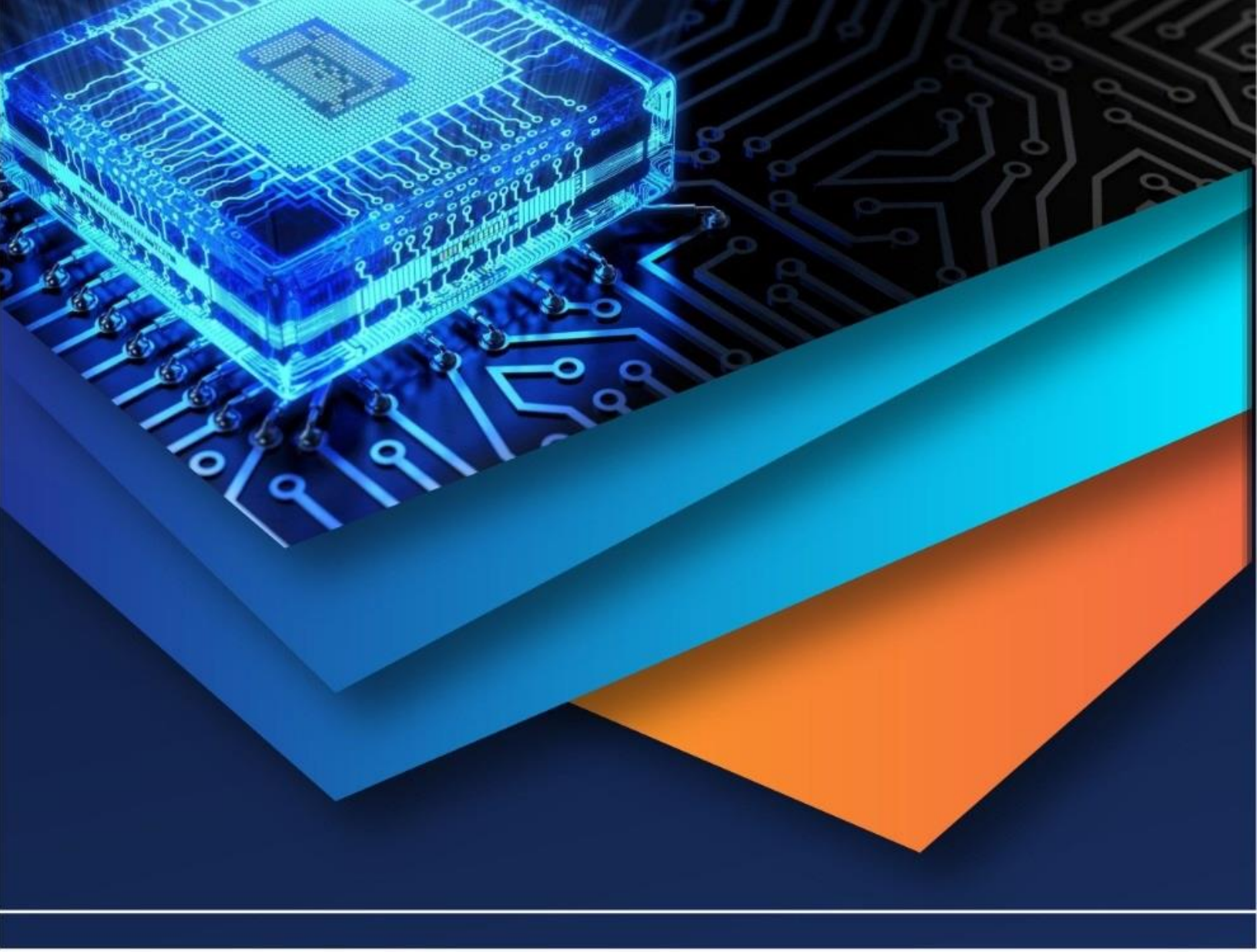

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