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Study ABO Blood Grouping of Forensically Important Saliva Samples through Assessment of Surface Materials-an Effective Parameter.

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Abstract: ***Detection of saliva stains encountered in forensic investigation is one of the primary objectives for forensic serologist as saliva may be an important source of DNA. Blood grouping can be done from the saliva if the person is secretor in status. Saliva is one of the vital fluids secreted in human by salivary glands, which may be deposited on the human skin through biting, sucking, licking, kissing, and possibly through other behaviors and often found in various types of crime scenes, such as hanging, sexual harassment, rape, homicide, suicide. Saliva stains may be found on any surface at the scene of crime as vital evidence. In study saliva stains were prepared on different fabric materials and metals in the laboratory as such the mimic of crime scene was created. Blood grouping examination was done for the piece of different fabric materials between the time intervals of 24 hours. The present study was undertaken to find out the maximum duration for which blood grouping is possible for respective blood group when the stains are obtained from different fabric materials and metals and also to determine the affection on blood grouping by different surfaces of fabric materials.***
Keywords: Saliva, Crime Scene, Blood grouping.

I. INTRODUCTION

The majority of the oral fluid originates from three pairs of major salivary glands (gl. parotis, gl. submandibularis and gl. sublingualis). Other sources, responsible for the composition of the oral fluid, are the gingival crevicular sulci (area between tooth and marginal free gingiva), an estimated number of 450–750 minor accessory salivary glands, situated on the tongue, the buccal mucosae and the palate, and oro-naso-pharyngeal secretions. The complex mix of salivary constituents provides an effective set of systems for lubricating and protecting the soft and hard tissues. Protection of soft tissues is afforded against desiccation, penetration, ulceration, and potential carcinogens by mucin and anti-proteases. Saliva can encourage soft tissue repair by reducing clotting time and accelerating wound contraction. A major protective function results from the salivary role in maintenance of the ecological balance in the oral cavity via: (1) debridement/lavage; (2) aggregation and reduced adherence by both immunological and non-immunological means; and (3) direct antibacterial activity. Saliva also possesses anti-fungal and anti-viral systems. Saliva is effective in maintaining pH in the oral cavity, contributes to the regulation of plaque pH, and helps neutralize reflux acids in the esophagus. Saliva at crime scenes, on the victim, suspect, or witnesses (clothing or persons) can be considered significant and treated as such when documenting, collecting, and preserving investigator. It can link a victim to a suspect. Crime scene investigators could soon use saliva left behind at crime scenes to determine the ages of those involved at the scene. Saliva will also be of great significance if found at crime scene, such as on the victim of a sexual assault, on the cigarette end, or around the rim of a glass or bottle. Traditionally saliva at crime scenes has been documented and collected for identification, through Blood grouping if the person in secretor in status and DNA, at a crime laboratory.

II. MATERIALS AND METHODS

Saliva samples are the major requirement for the research study. Saliva samples were collected from volunteers of department of life science, Gujarat University, Ahmadabad with care and by the expert with consent. First of all secretors status of volunteer was checked then Saliva was collected on the basis of selected ABO secretor, such as A blood group antigen secretor, B blood group antigen secretor and AB blood group antigen secretor AB.

In almost all crime scenes when saliva found as evidence is in dried form. So as here in study the mimic of crime scene was created in laboratory. For the forensic analysis collected saliva was allowed to dry on different surfaces. For each sample preparation Different cloth materials such as Cotton, Silk, Linen, Georgette, Velvet & Jute were cut in pieces about 5cm and dipped in saliva and allowed to dry. Dried stains were prepared of Different blood groups. ABO blood grouping was done but cutting the stain about

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2mm long as followed by the procedure done in forensic investigation of real crime scene.

For other surface analysis such as Glass, Plastic, Steel, Metal, and wood the saliva was poured on surface of each specimen with marking and all the samples were allowed to dry at room temperature in Laboratory condition. After drying samples were collected by swab using Distilled water and then swab was allowed to dry. And then ABO blood grouping was done from that dried swab as done in forensic crime scene investigation.

For the soil analysis saliva thread about 5cm was prepared which was also dried and buried in collected soil in Petri plates.

A. Presumptive Test

Presumptive test was performed by Placing 3 tubes in a rack and followed by given method.3 drops of soluble starch solution was added to each tube. Mixed, cork and incubated the tubes for 1 hour at 37°C. then 2 drops of Lugol's iodine was added and color formation was noted down. A dark blue starch-iodine complex should be observed in the first and second tubes. The absence of the dark blue color indicates that the starch has been hydrolyzed and is no longer available for complexion. Therefore, the lack of blue color is a positive result for amylase activity, indicative of the presence of saliva. This is not a confirmatory test for saliva.

B. Absorption-illusion method

For this method two clean and dry test tubes for each individual sample were taken, marked as A and B. One extra tube was taken and marked as control. Then stained fabric, swabs and thraed were cut about 2mm long for each sample and were added in test tubes. Then one drop of antisera was added on basis of markings Anti-A was added in A and Anti-B was added in B test tubes for each sample. All tubes were allowed to put in refrigerator at 4°C overnight. Next day all tubes were washed by chilled normal saline to remove excess antisera. Washing was repeated 3-4 times. Then all tubes were plugged by cotton and incubated in water-bath at 50-60°C about 20 min for elusion as shown in fig 1. Then blood cells of blood group-A and blood group-B in respective tubes of A and B and in control any of them were added. Tubes were allowed to put in refrigerator at 4°C for 30 min. And result for agglutination was observed under light microscope. The method was repeated at the time interval of 24 hours for each sample till the results appear to be negative.

Fig.1 Samples kept for Elution at 50-60°C

III. OBSERVATION

24 Hours observation

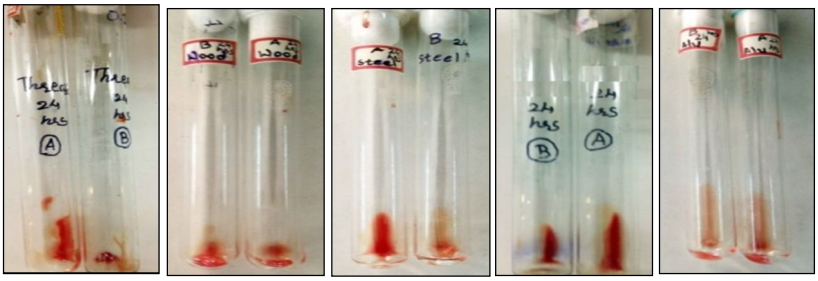

Fig.2 Tubes for Thread, Wood, Steel, Iron, and Aluminum respectively after adding saliva stains

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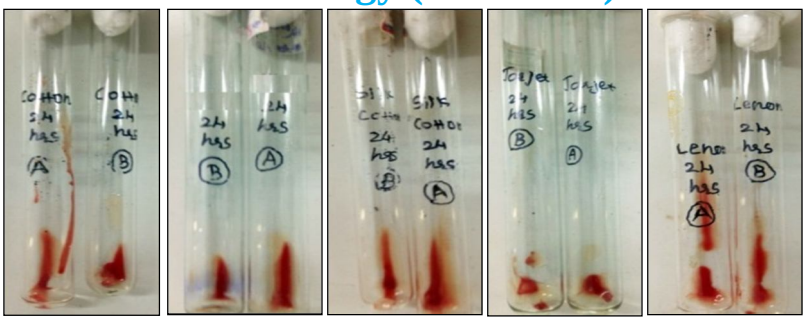

Fig. 3 Tubes for Cotton, Silk, Jute, Georgette and Linen respectively after adding saliva stains

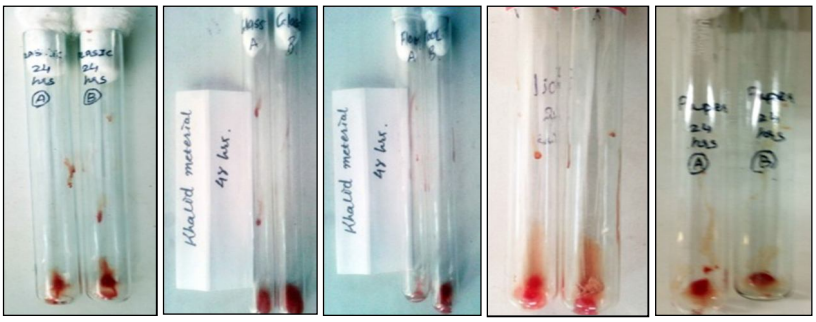

Fig.4 Tubes for Plastic, Cotton, Floor, Soil, Paper respectively after adding saliva stains

IV. RESULTS

TABLE-I:

ABO Blood grouping for Thread, Wood, Steel, Iron and Aluminum

Time (Hours)	Thread			Wood			Steel			Iron			Aluminum		
	A	B	O	A	B	O	A	B	O	A	B	O	A	B	O
24	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++
48	+++	+++	+++	++	++	+++	+++	++	++	++	++	++	+++	++	++
72	+++	+++	+++	++	++	+++	++	++	+	++	++	++	++	+	++
96	+++	+++	+++	++	++	++	++	++	+	+	+	+	+	+	++
120	+++	+++	+++	+	++	++	++	+	+	+	+	+	+	+	+
144	++	+++	+++	+	+	++	+	+	+	+	+	-	+	+	+

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168	++	++	+++	-	-	+	-	-	-	-	-	-	-	-	+
192	++	++	++	-	-	+	-	-	-	-	-	-	-	-	+
216	+	+	++	-	-	+	-	-	-	-	-	-	-	-	-
240	+	+	++	-	-	+	-	-	-	-	-	-	-	-	-
264	-	-	++	-	-	+	-	-	-	-	-	-	-	-	-
288	-	-	++	-	-	+	-	-	-	-	-	-	-	-	-

TABLE-II:
ABO Blood grouping for different cloth materials

Time(Hours)	Thread			Cotton			Silk			Jute			Georgette			Linen			Velvet		
	A	B	O	A	B	O	A	B	O	A	B	O	A	B	O	A	B	O	A	B	O
24	+++	+++	+++	+++	+++	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
48	+++	+++	+++	+++	+++	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
72	+++	+++	+++	+++	+++	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
96	+++	+++	+++	++	++	+++	++	++	+++	++	++	+++	++	++	+++	+++	+++	+++	++	+++	+++
120	++	++	+++	++	++	+++	++	++	+++	++	++	+++	++	++	+++	+++	+++	+++	++	++	+++
144	++	++	+++	++	++	+++	+	+	+++	++	++	+++	++	++	+++	+++	+++	+++	++	++	+++
168	++	++	+++	++	+	+++	+	+	++	+	++	+++	++	++	+++	++	++	+++	+	++	+++
192	++	++	+++	+	+	+++	+	+	++	+	+	+++	+	++	++	++	++	+++	+	++	++
216	+	+	++	+	+	++	+	+	++	+	+	++	+	+	++	++	++	+++	+	+	++
240	+	+	++	+	+	++	+	+	++	+	+	++	+	+	+	++	++	++	+	+	++
264	+	+	++	+	+	++	+	+	++	+	+	+	+	+	+	++	++	+	-	+	++
288	+	+	++	+	+	++	+	+	++	+	+	+	+	+	+	+	+	+	-	+	++

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312	+	+	++	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	++
336	+	+	++	-	-	+	-	-	+	-	-	+	-	-	+	+	+	+	-	-	++
360	+	-	++	-	-	+	-	-	+	-	-	+	-	-	+	+	+	+	-	-	+
384	-	-	++	-	-	+	-	-	+	-	-	+	-	-	+	+	+	+	-	-	+
408	-	-	++	-	-	+	-	-	+	-	-	+	-	-	+	+	+	+	-	-	+
432	-	-	++	-	-	+	-	-	+	-	-	+	-	-	-	+	+	+	-	-	+
456	-	-	++	-	-	+	-	-	-	-	-	+	-	-	-	+	+	+	-	-	+
480	-	-	++	-	-	+	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-
504	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
528	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE-III
ABO Blood grouping for Plastic, Glass, Stone floor, Paper

Time	Plastic			Glass			Stone floor			Soil			Paper		
(Hours)	A	B	O	A	B	O	A	B	O	A	B	O	A	B	O
24	++	++	+++	++	++	+++	++	++	+++	+++	+++	+++	+++	+++	+++
48	++	+	++	+	+	+++	++	++	++	+++	+++	+++	+++	+++	+++
72	+	+	++	+	+	++	+	+	++	++	++	+++	+++	+++	+++
96	+	+	++	+	+	++	+	+	+	+	+	++	+++	+++	+++
120	+	+	+	-	-	++	-	-	+	+	+	++	+++	+++	+++
144	-	-	+	-	-	++	-	-	-	+	+	+	++	++	+++
168	-	-	+	-	-	+	-	-	-	-	+	+	++	++	++
192	-	-	+	-	-	+	-	-	-	-	-	+	++	++	++
216	-	-	+	-	-	-	-	-	-	-	-	+	+	+	++
240	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
264	-	-	-		-	-	-	-	-	-	-	-	+	+	+
288	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
312	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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V. DISCUSSION

From the study of different forensic materials for the ABO blood grouping from saliva we conclude that different material surfaces give different grouping results. As the blood grouping was done on different materials surface with time period of 24 hours as shown in the observation table-I. The results got negative at different time period on each material. In wood at 24 to 48 hrs result was so clear and visible for blood group-A and blood group-B, while 24 to 72 hrs for blood group-O. At 72 to 96 hrs it goes slightly visible for blood group-A and blood group-B, while 96 to144 hrs for blood group-.O. After that till 144 hrs for blood group-A and blood group-B, and till 288 hrs for blood group-O it was visible under microscope. This shows that the wood contains termite and other micro-organisms which may affect the salivary antigen so that result decreases and at 168 hrs result got negative for blood group-.A and blood group-.B. In Steel from the beginning only till 48 hrs for blood group-A, till 24 hrs in blood group-B while less than 24 hrs for blood group- O the results were clearly visible. After that the visibility decreases to slightly visible from 72 to120 hrs for blood group- A, 48 to96 hrs for blood group- B and 24 to 48 hrs for blood group-O. At 144 hrs results were visible under microscope for blood group-A, 120 to 144 hrs for blood group-B and 72 to144 hrs for blood group-O. And from 168 hrs onwards results got negative for all the three blood groups. Which shows that salivary component may react with steel surface's molecules which may affect salivary antigen so grouping cannot be done for longer period. From the observation table I we can conclude that in Iron when grouping done at 24 hrs result obtained was visible clearly in tubes. Then till 72 hrs the visibility slightly decreases After that till 144 hrs result obtained was visible through microscope only and then from 168 hrs result got negative. From these results we can conclude that iron surface effect on salivary antigen of the saliva so blood grouping does not get long time. In Aluminum at 24 and 48 hrs result were clearly visible in tubes for blood group- A and only till 24 hrs for blood group- B and blood group- O. After that results decreases in blood group- B and blood group- O was visible only in microscope, and in A blood group result remains same at 72 hrs while in blood group-O it was visible till 192 hrs. At 168 hrs result got negative in blood group-A and blood group-B, and 216 hrs in blood group-O. From these results we can conclude that aluminum surface affect salivary antigen of Blood group-B more than blood group-A and blood group-O.

Observation table-II shows blood grouping done on thread, cotton, silk, jute, georgette, linen and velvet. From the table II we can conclude that in Cotton cloth material when grouping done from 24 hrs to 72 hrs for A and B blood groups, and till192 hrs for O blood group the results are visible in tubes after that at 96 to 168 hrs for blood group-A, 96 to 144 for blood group-B and 216 to288 for blood group-O results decreases to slightly visible. The results decrease visible only microscopically from 192 to 312 hrs for blood group-A, from 168 to 312 hrs for blood group-B and 312 to 504 hrs for blood group-O. Results got negative in both at 336 hrs and at 528 hrs for blood group-O. In Silk cloth material results are visibly good from 24 to 144 hrs for blood group-O as compared to A and B blood groups. From 24 to 120 hrs for both A and B blood groups, and 168 to 288 hrs for blood group-O the results are slightly visible in tubes. At 144 till 312 hrs results visible only at microscopically for blood group A and B, and at 312 till 432 hrs for blood group-O. At 336 hrs results got negative in both A and B blood groups and 528 hrs for blood group-O. In Jute from 24 to 72 hrs for A and B blood group and 24 to 192 hrs for blood group-O results are visible in tubes after that results decreases to slightly visible at 96 hrs to 144 hrs for A blood group-A, 96 to 168 for B blood group-B and 216 to240 hrs for blood group-O. There after the result got visible only microscopically till 312 hrs for A and B blood group and at 264 to 480 hrs for blood group-O. At 336 hrs results got negative in both A and B blood groups, while from 504 hrs. In Georgette cloth material from 24 till 72 hrs results visible in tubes for A and B blood group and till168 hrs for blood group-O . After that results decreases to slightly visible at 96 to 168 in blood group-A till 192 hrs in blood group-B while for blood group-O it is till 216 hrs. At 192 hrs results in blood group-B remains same and results decreases to microscopically visible in blood group-A till 312 hrs. At 216 hrs results decreases in blood group-B visible only at microscopically till 288 hrs and 240 to 408 hrs for blood group-O. At 336 hrs results got negative in both A and B blood groups while for blood group-O results get negative from 432 hrs onwards. In Linen cloth material from 24 to 144 hrs results are visible for A and B blood group and till 216 hrs for blood group-O. After that from 168 to 264 hrs the result becomes slightly visible for A and B blood groups and 240 hrs for blood group-O. At 288 to 480 hrs results decreases to only microscopically visible for A and B blood groups while for blood Group-O it is 264 to 504. At 504 hrs onwards results got negative for A and B blood groups and from 528 hrs for blood group-O. In Velvet cloth material from 24 to 72 hrs in blood group A, till 96 hrs for blood group-B and till 168 hrs for blood group-O results are visible clearly in test tubes. After that the result becomes slightly visible from 96 to 144 hrs for blood group-A, 120 to192 hrs for blood group-B and 192 to336 hrs for blood group-O. At 168 to 240 hrs results decreases to only microscopically visible for blood group-A, from 216 to288 for blood group B and 360 to456 for blood group-O. From 264 hrs onwards for blood group- A 312 hrs for blood group-B and 480 hrs onwards for blood group-O results got negative.

Observation table III shows blood grouping on plastic, glass, stone floor, soil and paper. In Plastic the results are clearly visible at

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24 hrs for blood group- O as compared to blood group- A and blood group- B where the results are only slightly visible in tubes and which continue till 48 hrs for blood group- A while till 96 hrs for blood group-O. And after that results decreases to visible only microscopically at 72 to 120 hrs for blood group-A and blood group-B while 120 to216 hrs for blood group-O. At 144 hrs results got negative for blood group-A and blood group-B and at 240 hrs for blood group-O. In Glass surface at 24 hrs results are clearly visible in blood group-O whereas it is slightly visible in tubes for blood group-A and blood group-B. After that at 48 to 96 hrs result is visible only in microscope for blood group-A and blood group-B and till 192 hrs for blood group-O. At 120 hrs results got negative in both tubes of blood group-A and blood group-B while from 216 hrs onwards for blood group-O. From these results we can conclude that glass surface affect salivary antigen more than other surface. In Stone floor when grouping done at 24 and 48 hrs result slightly visible in tubes as compared to blood group-O and after that at 72 and 96 hrs results decreases to visible only microscope in blood group-A and blood group-B which remains same till120 hrs in blood group-O . At 120 hrs results got negative in both tube and at 216 hrs for blood group-O. In soil at 24 and 48 hrs results are clearly visible in tubes for A and B blood groups which continue till 72 hrs for blood group-O. At 72 hrs results decrease to slightly visible for both A and B blood groups while from 96 to 120 hrs for blood group-O. At 96 to 144 hrs result visible only microscope in blood group-A and 96 to168 hrs in blood group-B while for blood group-O it remains till 216 hrs. At168 and 192 hrs got negative result for blood group-A and blood group-B respectively while at 240 hrs onwards for blood group-O. From these results we can conclude that in soil contains diverse and large amount of microorganisms so that they may decompose salivary component. In Paper when grouping done at 24 to 120 hrs result were clearly visible in tubes for both A and B blood groups while for blood group-O it remains same till 144 hrs. After that at 144 to till 192 hrs results decreases to slightly visible for A and B blood groups whereas 168 to 216 hrs for blood group-O. At 216 to 364 hrs results decreases to only visible microscopically and got negative at 288 hrs for blood group-a and blood group-B while it remains microscopically visible for blood group-O from 240 to288 hrs and then got negative.

VI. CONCLUSION

Forensic investigation of saliva has now become a significant part of any crime investigation. Saliva is very useful evidence to identify an individual. Most of the time saliva found at crime scene in very small quantity through its potency is very high. If saliva is affected by any circumstances then it is difficult to identify individual or person involved in crime. As saliva may be found on any cloth material either from suspect or victim so the study signifies the time period up to which blood grouping can be done. And in some situation predict that how to done crime and how much time before it done. Saliva may also be found on weapon and it can be of any material such as iron, steel, wood, glass, aluminum, plastic, stone, etc. So the study signifies the effect of different surfaces on blood grouping which vary from one to another. The study mainly signifies to prevent the false interpretation of the blood grouping examination during forensic investigation.

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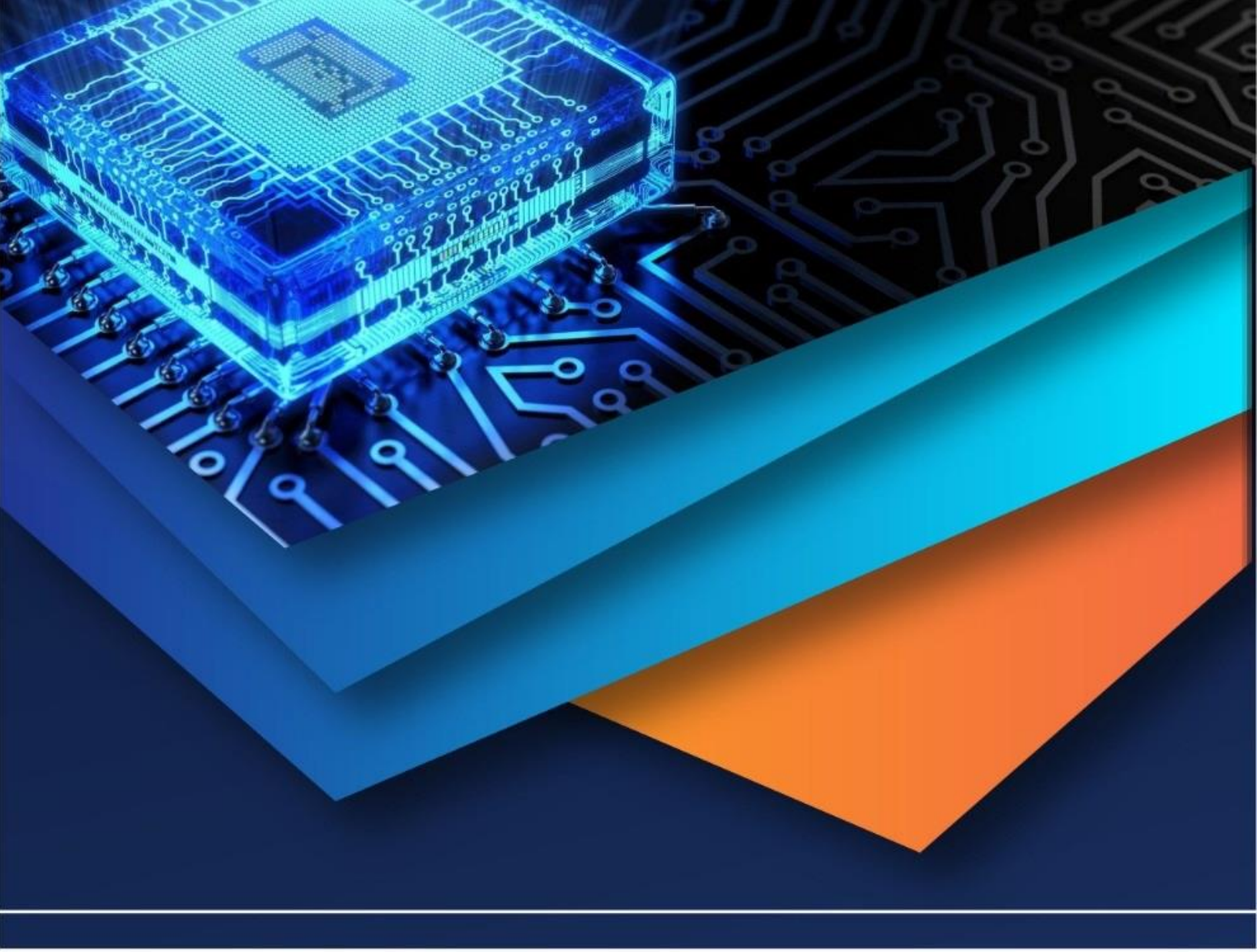

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