Original Research

Forensic Serological Study of Degradation of Antigenic Properties in Human Blood

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Abstract

Forensic Serological analysis involves various examinations of exhibits that are presented to the Forensic Science Laboratory, by the investigating agencies. It is often found in various states of degradation. The presumptive tests will indicate positive results; however, the further testing is impaired due to degradation of the various protein factors, due to the exposure in various environmental conditions that prevail at the scene of occurrence or places where blood laden evidence is deposited. This study undertakes to simulate conditions and examine the degradation of the antigenic properties of blood.

The study was undertaken with the methodology of storing the collected blood samples in laboratory conditions, simulated conditions such as smear on twigs, razor, knife, immersed in water and buried in soil. The samples were periodically removed and subjected to testing for the antigenic properties. The results obtained were discussed and with the help of the reviewed literature, matched for validation. This study is purposed to pave way for many more research among the student and researchers in Forensic Serology.

***Keywords**:* Analysis; Antigenic; Blood; Environment; Serological; Simulated condition

Introduction:

Blood is the most important body fluid encounter at the scene of crime, involving violence. The criminal acts range from homicidal attacks, grievous hurt and as well as sexual assault. Blood is often the lead source for the investigating team to tie the assailant to the *corpus delicti* and the crime scene. In a majority of the circumstance, blood is found in a coagulated condition on clothing, weapons, debris in the environment and soil. It is rarely fresh and in various stages of degradation, when it is forwarded to the laboratory for analysis, identification, and individualization.

Blood is made up of plasma and blood cells. Plasma is the major part and is composed of water, glucose, proteins, enzymes, hormones along with the blood cells suspended, similarly blood group antigens are either sugars or proteins, and they are attached to various components in the red blood cell membrane. For example, the antigens of the ABO blood group are sugars. They are produced by a series of reactions in which enzymes catalyze the transfer of sugar units. The antigen present in the blood determines a person's blood group. In contrast, the antigens present on the Rh blood group are proteins. The RhD gene encodes the D antigen, which is present in few and absent in few. This study is on the degradation of antigenic properties under proposed conditions with ABO typing[1].

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Objectives:

- To determine the rate of degeneration of antigenic properties of blood in a specified duration of time, the time gap being in geometric progression.
- To study the interference of different antigens in blood grouping over a specified duration of time and to check the strength of blood stains.

Hypothesis:

The hypothesis formed for the purpose of this study is as given under:

- The samples subjected for analysis show degeneration of antigenic properties with increase in the number of days they are subjected to the parameters.

Materials and Methods:

Materials:

1. For collection of blood samples: Sterile gauze cloth, lancets, surgical spirit, etc.
2. For exposure to different parameters: Petri Dishes, Moist soil water, twigs, rusted razor and knife.
3. For detection of origin of species: Agarose gel, punching instrument, conical flask and other glassware.
4. Instrumentation in grouping and origin: Centrifuge, heater, refrigerator, and laboratory research microscope.

Sample Selection

The study was conducted at Regional Forensic Science Laboratory Mysore, Karnataka. The method of sampling selected is "random sampling". The samples were collected from subjects belonging to age group between 15-60 years. The subject belonged to both the sexes. The geographical area covered for sample collection was throughout Mysore city. 40 subjects were selected. These subjects selected were informed about the study and blood was collected with their consent. As a control sample, 5 liquid blood samples (1 each belonging to A, B and AB groups and 2 samples belonging to O groups) were collected from the blood bank of K. R. Hospital, Mysore.

40 blood samples were collected on sterile gauze cloth, dried and placed in plastic zip pouches and labeled. In the laboratory these samples were placed in petri dishes and labeled. These samples were then maintained at two different conditions ie., laboratory condition, and exposed to sunlight All of these samples were maintained at Regional Forensic Science Laboratory (RFSL), Mysore. The samples were subjected for analysis on 2^{nd}, 4^{th}, 8^{th}, 16^{th} and 32^{nd} day at RFSL, Mysore.

The above parameters were chosen on the grounds that the articles encountered by the Forensic experts in routine cases[2].

Exposure Method:

- For laboratory condition: First 10 samples were placed one each in 10 petri dishes, labeled from L1-L10 and maintained in the laboratory as control samples.
- For exposure to sunlight: The next 10 samples were placed one each in 10 petri dishes, labeled from N1-N10 and placed on the terrace of RFSL, Mysore.

For determination of origin of species using gel diffusion method:

- **Preparation of the extract:**

The blood-stained samples are placed in test tubes and labeled.

  - Few drops of saline are added so that the samples get soaked in it and allowed to stand for 2-3 hours.
  - The test tubes are thoroughly shaken on a vertex to get the extract and the extract is diluted if necessary.

- **Preparation of agar plate:**
  - 1 g of agar powder is added to 100 ml of water and stirred well.
  - The beaker is then heated with occasional stirring to melt the agar powder.
  - Heating is stopped when 1^{st} vigorous boiling of water takes place.

- It is allowed to stand for 2-3 min so that the foamy bubbles disappear. The melted agar is then poured on glass slab of 2*3 inch or on petri dish to form a layer of about 2 mm.
- The glass slab or petri dish is allowed to set for 5-10 min. Once the agar has solidified the plates are refrigerated.
- Wells are punched into the plate whenever necessary.

- **Loading the plate:**
  - With the help of micro-pipette the extract is placed in the wells of agar plate leaving the central well empty in each set where the human anti-sera is added.
  - The plate is refrigerated for 19-20 hours. (in case of fresh stains results are obtained within 5-6 hours).
- The plates are then observed against light.
- If a faint white arc is observed between the well containing human anti-sera and the well containing the sample extract, the sample is said to be of human origin. If no white arc is observed, the sample is not of human origin.

Observations:

Results of blood grouping using absorption elution technique and origin of species tests:

I. Samples maintained at laboratory condition:

- Only the second day samples showed true result but with a low degree of agglutination.
- The 4th and 16th day samples belonged to group B. they showed strong positive result for group B. however, there was interference by A and H lectins.
- The 8th and 32nd day samples also belonged to group B and showed false positive result.
- All samples were positive for Origin of species test.[3]

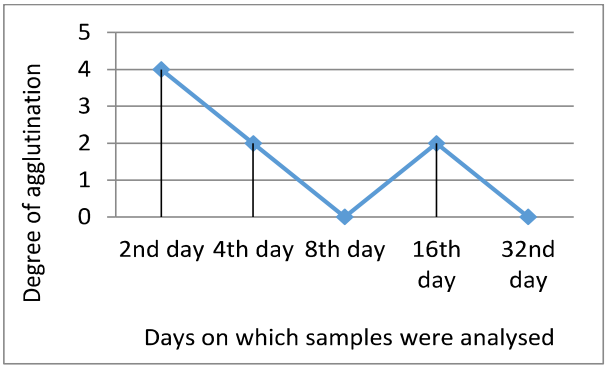

Figure 1. Chart representing degree of agglutination in laboratory samples.

II. Samples exposed to sunlight:

- The 4th and 8the day samples showed true result. The 4th day sample showed low degree of agglutination, whereas, 8th day sample showed very high degree of agglutination.
- The 2nd and 16th day samples belonged to group A and group B respectively. These samples showed positive results, but with a comparatively low degree of agglutination.
- The 32nd day samples belonged to group O and showed very low degree of agglutination. This is taken as no result.
- The 8th and 16th day samples were positive for origin of species test, whereas, 2nd, 4thand 32nd day samples were negative.

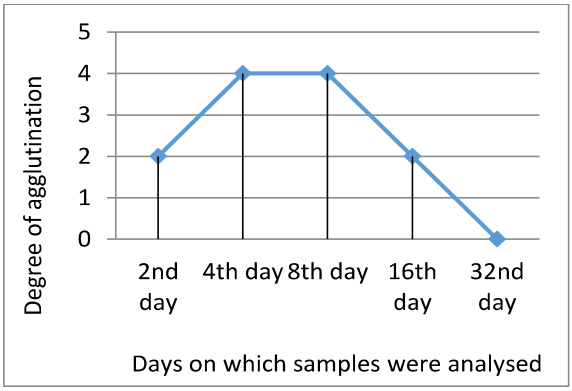

Figure 2. Chart representing degree of agglutination in samples exposed to sunlight.

Overall interpretation of results:
For origin of species:

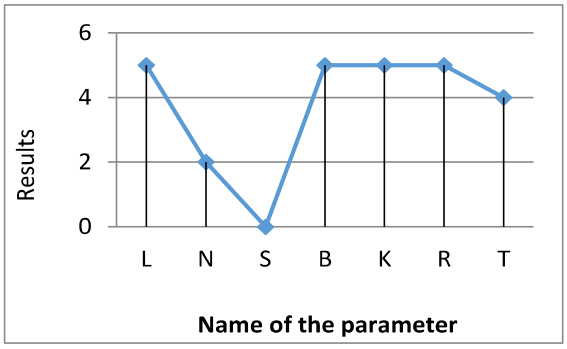

Figure 8. Overall result for origin of species.

Factors affecting the antigenic properties of blood:

There are several factors affecting the integrity of stains and properties of blood. These factors can greatly interfere with the tests held at the Forensic Science Laboratory. They may lead to false grouping and origin which can have an adverse effect on the related cases. The following factors were observed.

- **Environmental temperature:** Here environmental temperature refers to the temperature to which the blood is exposed for a considerable period. The rate of degeneration of properties of blood is greatly accelerated et lower temperature.
- **Humidity:** A blood sample kept at humid conditions will dry much slower than samples kept at dry conditions. It should be borne in mind that dry materials also contain water. Proteins bind water very tenaciously.
- **Sunlight:** Sunlight can cause deterioration of properties of blood by causing denaturation of proteins in it. Sunlight is mainly composed of heat and light energy. These samples also showed progressive fall in the antigenic properties, but with no interference from other antigenic and there was no false positive result. The samples exposed to sunlight were kept closed in petri-dishes. Hence, although the temperature was very high (beyond 35^{o}C) the closed chamber might have prevented the complete loss of moisture, which in turn reduced the shrinkage of blood cells. Hence, the antigens were not completely lost and facilitated grouping.
- **Age of stains:** At the scene of crime the age of stains can be determined based on the colour of stains. Bright red colour indicates fresh bloodshed. Reddish-brown colour indicates a time lapse of 24 to 36 hours. Dark brown colour indicates that the blood was shed about 2-3 days before. Dark brown or blackish brown colour indicates a time lapse of more than 3 days.
- **Endogenous degradable enzymes:** Blood plasma contains a number of endogenous degradable enzymes mainly associated with coagulation system. These proteases will be activated in freshly shed blood and may act on some of the plasma protein markers in un-lysed blood and both plasma and intracellular markers in lysed blood.
- **Laboratory condition:** The samples maintained in this condition showed a progressive fall in the antigenic properties. Only the 1st day sample showed positive result, but with low degree of agglutination and there was no interference by other antigens.

The observations made in this study are also affected by various other factors, such as:

- Anti-sera.
- Storage devices.
- Instruments used.
- Equipment used.
- Skill of the person conducting the tests.

Interpretation of result with respect to hypothesis and conclusion:

Considering the hypothesis: “The samples subjected for analysis show degeneration of antigenic properties with increase in the number of days they are subjected to the

parameters." The samples have shown degeneration in their antigenic properties, but the graph is fluctuating and does not show any precise trend. However, the samples maintained at laboratory condition and those exposed to sunlight have shown a somewhat increase in the rate of degeneration. The hypothesis holds true only for these 2 parameters. This is also found in a research conducted by A.A., El Habashi et al[3], studied the factors affecting ABO grouping of dry blood stains in Riyadh, including exposure to extremes of temperature, from refrigeration at -4C up to heating at 15^0C, effect of time till six months, occurrence of the stains on different fabrics, and effect of putrefaction. From their study it was suggested that Chromatographic separation was a suitable procedure before grouping the samples which were buried in sand or mud. In another study by M. Hara et al[4], the effects of various storage conditions on blood identification tests, DNA degradation, and short tandem repeat (STR) typing were evaluated. Bloodstains stored at room temperature, 4 °C, -20 °C, and -80 °C for 20 years; blood samples stored at -20 °C and -80 °C for 20 years; and fresh blood samples were analyzed. Leuco-malachite-green testing, anti-human haemoglobin (Hb) testing (using immunochromatography), and tests for hemoglobin-beta (HBB) mRNA were performed as blood identification tests. DNA degradation was evaluated by quantifying the ratios of 305 and 129 base pair (bp) fragments to 41 bp fragments. STR typing was performed using an AmpFlSTR® Identifiler™ Plus PCR Amplification Kit. Their study revealed that to prevent DNA degradation during long-term storage, it is recommended that bloodstains and blood be stored at below -20°C. In addition, because bloodstains are more suitable for detection of blood-specific mRNAs than blood sample, it is desirable that blood is stored as bloodstain.

Further, Randel H Abdel Hady et al, in their study experimented on Blood and seminal stains exposed to temperature grades 100°C, 50 °C, and burn show marked reduction in DNA concentration while maximum DNA concentration could be recovered from stains exposed to temperature grade temperature. Both blood and seminal DNA was affected only in case of burn without significant difference between THO1 and Amelogenin primers. They concluded that high environmental temperature affects the quantity of extracted DNA from different stains but less effect on the quality of extracted DNA. Burn affects both preliminary test, DNA quantity, and quality in stains. The most challenging part when it comes to forensic examination is regarding the storage.

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Conflict of Interest: None to declare

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