Effect of Ageing and Environment of North Maharashtra on Abo Grouping Substances of Blood Stain

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ABSTRACT

Blood stain can be crucial in reconstructing crime events. Environmental factors such as temperature and humidity will affect on Blood group system. Warm temperature will facilitate drying and cold temperatures will retard the drying times. Humidity also affects on blood stain, high humidity environments will dry more slowly than low humidity environment. High humidity also influences growth of bacteria and fungi while low humidity has reverse effect. Morphologically this region has pleasant climate almost throughout the year, pollution is less, and mean annual temperature is 20° C. Annual rainfall is 650 mm and humidity is more than 70%. In the present study an attempt was made to understand the extent to which environmental conditions effects on blood stain in North Maharashtra region including exposure to different temperature 4°C, 56°C 110°C and effect of time span till 2 years.

Keyword: Blood stain; age determination; environmental factors; ABO group system

INTRODUCTION

It had been observed that on white cloth the fresh blood stains are of bright red color. After a variable interval the color gradually changes to reddish-brown within about 24 hours and due to conversion of hemoglobin into methaemoglobin and haematin brown within a few days, which may become black after a long time [1, 2]. In criminal investigation the evidence of blood is almost in the form of dried blood stains. It is therefore necessary to understand what happens on drying. The first change that takes place in blood on exposure is clotting of blood. Further, complex changes take place on drying1. The cell structure is often destroyed. The protein loses some of their activities. Bacteria, fungi, heat and light may
bring about other changes. In dried blood stains as the red blood cells are haemolysed, and difficult to do their grouping. In a crime scene investigation, in the absence of fresh blood, dried blood stains are taken for analysis. In these dried blood stains, the red blood cells are all haemolysed. Though in such cases, the grouping may difficult. However, the blood group antigen contained in the haemolysed cells especially and ABO antigen has been known to retain their property of combining with specific antibodies for a considerable period of time [3].

Present work has been taken up to study the effect of temperature and ageing of blood stain on ABO grouping substances in order to demonstrate their importance as an important marker in forensic case work [4].

**PROCEDURE**

A) Blood stain samples were collected from storage of biology division. These prepared blood stain samples were maintained under five different conditions:

1. First set of blood stains were kept at room temp.
2. Second set of the samples were kept at 4 °C.
3. Third set of stains were kept at 37 °C.
4. Fourth set of sample were maintained at 56 °C.
5. Fifth set of samples were kept at 110 °C.

These stains were examined for ABO grouping. The analysis was performed by Absorption Elution Method [6, 7]

Specific antibodies are allowed to absorb on blood stain fibers and allowed to elute bound antibodies, indicator cells are used to eluted antibodies. Specially designed fiber washing tubes are used to remove unbound antibodies. Blood grouping of the blood stains were performed by absorption elution technique as follows.

(a) Three cleaned and dry test tubes were taken and marked them A, B and H.
(b) Blood stained cloth was cut about 2sq. mm to 5 sq.mm long threads were taken in each test tube. These test tubes were kept in an oven at 100 °C for 30 minutes.
(c) Fibers were dipped in anti A serum, Anti B serum and anti H lectin respectively and kept at 40 C for overnight.
(d) Fibers were washed in washing tubes and given 3-4 washing with ice chilled normal saline.
(e) Add one drop of 0.2 to 0.5 % A, B and O indicator cells in cavity slide respectively.
(f) fibers were put into two cavity slides labeled A,B,H and kept in oven at 560 C for 15 minutes in incubator after that kept slides at 40 C for an half hour.
(g) Centrifuge shake and examined the contents for agglutination macroscopically and microscopically under 10X resolution.

**Microscopic agglutination**

+++ : Very large agglutination clumps with few free cells.
++ : Smaller agglutinates with more free cells.
+ : Agglutinates of 5-10 cells with many free cells.
W: Agglutinates of 3-5 cells with many free cells.
N: No agglutination.

B) 90 blood samples were taken for ABO blood grouping for interval of time since last two years. These stains were examined for ABO grouping by Absorption Elution Method.

**RESULTS AND DISCUSSION**

Stains stored at room temperature, 40 c and 370 c gives same agglutination strength up to two years while stains stored at 560 C gave same agglutination strength up to nine month then degree of agglutination was decreases. After twentieth month these stains gave negative results. Stains stored at 110 °C gave same agglutination strength up to three month then degree of agglutination was decreases.
After Twelve month these stains gave negative results (Table 1).
We also observed agglutination strength for A, B, and O antigens stains. A and B antigens stains gave positive result up to two years and ‘O’ antigen gave positive result up to one year. After one year ‘O’ antigens gives negative result [8] (Table 2).

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<th>Table: 1 showing results of agglutination for different stains</th>
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<td>Age of stain</td>
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<td>Fresh</td>
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<td>Above twenty four months</td>
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<th>Table 2: Showing the results of A, B, O Blood groups</th>
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<td>Age of Stain</td>
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<td>Above twenty four months</td>
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CONCLUSION
Dried blood stains those kept at room temperature, 4 ⁰C and 37 ⁰C does not loses antigen activity up to two years. Stains stored at 56 ⁰C and 110 ⁰C were loses antigen activity after nine month. A and B antigens stains activity remain up to two years while ‘O’ antigen activity remain up to one year. A and B antigens stains loses antigen activity after about two years while ‘O’ antigen stains loses activity after about one year.
North Maharashtra environment [9, 10] is kept well A and B antigens stains up to two year while ‘O’ antigens stains for one year. The study demonstrate that a meaningful and more reliable results for A and B group stains for two years and O group stains for one year when they were stored at lower temperature as
compared to other conditions and it would serve as useful identifying marker for dried blood stain irrespective of temperature and age.

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CONFLICT OF INTEREST STATEMENT
The authors declare that they have no competing interests.

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