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Identification and Individualization of Blood on Burnt Cloth Debris

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Abstract: ***Blood and Bloodstain is most important and common form of the biological evidence which found on scene of crime. The identification of the blood or bloodstain on burned cloth debris is challenging in forensics for controlling the compliances with burning followed by murder, to destroy the crime scene or evidences. The aim of the study was to detection and genetic profile on burnt cloth debris where the origin of species and blood grouping giving inconclusive results. Thus it becomes essential to determine the sensitivity of reagents for crime scene management. In this study 6 different blood samples were collected and transferred on 6 different cloth materials were taken for classical biological or serological methods but the DNA profiling make the proper individualization of bloodstain from burned cloth debris. So it is necessary to examine the blood on burnt cloth debris in enhanced specially for DNA examination.***

Keywords: Evidence, Cloth Debris, False Positive, DNA profiling, Luminol

1. Introduction

Blood is a fluid connective tissue having cellular part and plasma. The pH of blood is slightly alkaline i. e.7.4. Blood cells are of two types - Erythrocytes (RBC) and Leucocytes (WBC). The WBC forms the immunoglobulin constitutes immune system. The erythrocytes are colored which make its distinguished feature from other body fluids. As the forensic aspect the blood and bloodstain are the most common type of evidence found at most of the crime scene. It is important in medico - legal practices help in solving crime of violence, suicide and accidents. It is a objective of a serologist/ biologist to identify whether the liquid/ stain is blood or not, is it human blood or non - human, what is the blood group of this blood/ blood stain forensic scientist uses preliminary tests or screening for the identification of blood. Tetra Methyl Benzidine, Kestle - Mayer test or Phenopthalein test and Luminal test. Luminol can detect the blood on washed surfaces, even under the few paint layers as well as in old samples. All the work on the activity occurs because of peroxidase enzyme produces energy in the form of light based on blue light emission. It is most important and well known assay in forensic science. The interferences of luminol reaction can be noticed in serological as well as DNA analysis. (**Brani, F., Lewis et al**).

DNA analysis was introduced to the criminal justice system in 1986, and since then there have been several improvements in the forensic technology and commercial products for short tendom repeat (STR) multiplexing from minute levels of template DNA. The technique of individualization from DNA analysis is becoming convenient with the passage of time, but it is difficult when deal with contaminated or degraded or specially treated or destroyed evidences. It is a topic of concern in the field of forensic science to identify the blood on the burnt surfaces it may present on various surfaces like cloth, paper, furniture and other house hold objects. Researchers studied the effects of fire or heat on bloodstain pattern analysis. They were created in typical household objects and furnishing. The structures were burned and extinguished by fire - workers using water, cooled overnight and then examined under bright light. Soot the physical barrier, removed by water or alcohol. They used preliminary examination of blood then DNA analysis. The recovered DNA seems unaffected (**Tontarski K. L et al**).

To determine the quality, quantity and amplifiability of extracted DNA from pig muscle and bone marrow to controlled burn on open flame or convection oven with varied time ranges. They result the low molecular weight, degraded DNA with undetectable yields **(Kadunc, R. et al**).

The recovery and detection of possible blood and bloodstain at the scene of crime can make a case advent and perfection of DNA testing. Typically, only the stains which yield a positive result with blood screening tests are collected for the subsequent DNA analysis.

2. Materials and Methods

The material of the study consist six different cloth materials i. e. Cotton, Pure Silk, Georgette, Khadi, Banarsi and Cotton mixed silk (Table 1). The blood samples were transferred to make stain. After one month the samples were burned in flame then it was cooled down in normal condition. After two month the tests were performed for the detection of blood and blood grouping after two weeks of burning. After another one week the genomic DNA was isolated from the burned cloth debris. The isolated DNA was amplified by polymerase chain reaction (PCR) and genotyping was done using 24 Short Tendom Repeats (STRs) markers.

Table 1: Cloth material taken as sample

S. NO.	*Samples*	*Cloth Material*
1.	S - 1	Georgette
2.	S - 2	Banarsi Silk
3.	S - 3	Cotton
4.	S - 4	Pure Silk
5.	S - 5	Khadi
6.	S - 6	Cotton mixed Silk

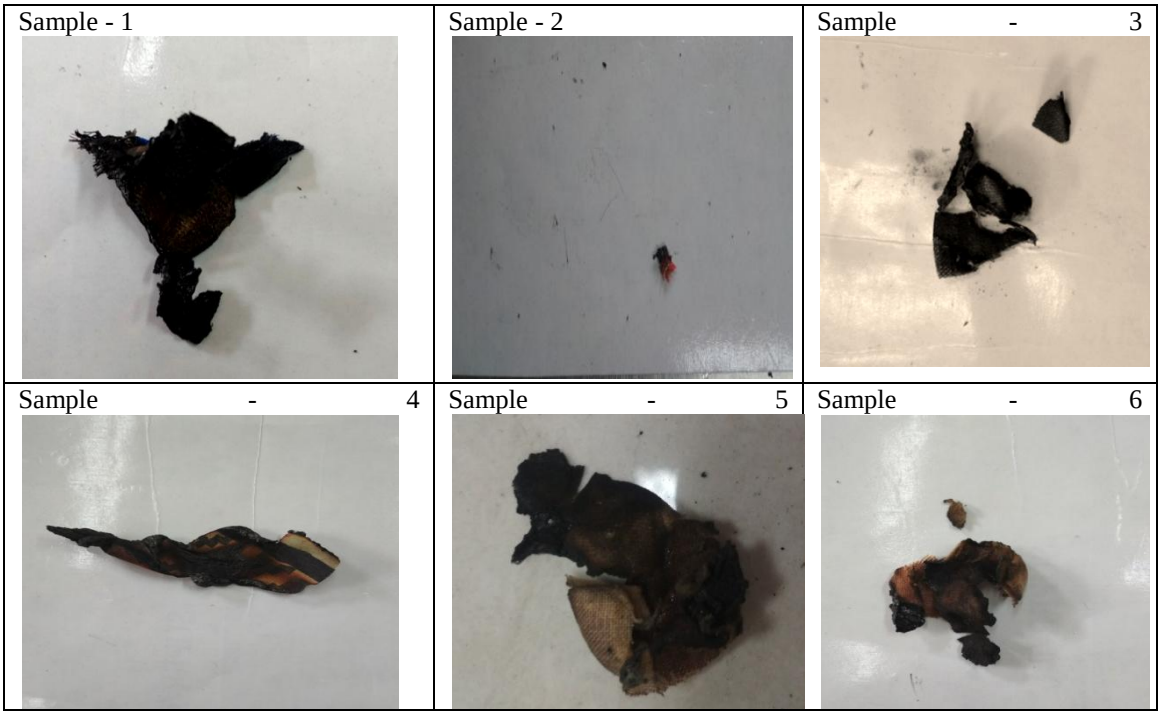

Figure 1: Burned cloth debris taken as samples

Detection of Blood: The small portions of burned cloth debris was taken and examined by Tetra Methyl Benzidine (TMB) as well as luminal solution for the detection of blood.

Origin Test: The extraction was done in 5% ammonia solution and the origin of species was performed by Ouchterlany method in agrose medium.

Blood Grouping: The burned cloth debris was analyzed for the detection of ABO blood grouping by absorption - elution method.

DNA Isolation method: Small parts of burned cloth debris were taken for the DNA isolation. The genomic DNA was recovered by the organic extraction method using QIAamp[at]Kit method according to the manufacturer's protocol.

Quantitation: The quantity of isolated DNA was checked by 1% agrose gel with standard ladder visualized under UV fluorescence.

PCR: PCR was carried out in Global filer Amplification kit followed by manufacturer's protocol in PCR system AB Veriti.

Genotyping: The genoptyping was done for autosomal 24 STR loci D3S1358, vWA, DI6S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, THO1, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338 including 3 sex determining markers namely Y - indel, Amelogenin and DYS391 PCR products were amplified in gene sequencer Applied Biosystem 3500 using the protocol of Globalfiler. The resultant allele distribution for the studied loci is showed in Table3.

Analysis of STR data: The amplified genetic sequence of the samples was analyzed by using Gene Mapper ID - X 1.6 Software.

3. Result and Discussion

The samples were examined for the identification of blood by TMB and luminal spray reagent. TMB produces faint bluish green color while Luminol emit blue chemiluminescence in dark. The glow only lasts about 30 seconds so it is photographed, then the samples were allowed for the origin test where no origin detected while precipitin band was observed in control sample. The samples were examined for blood grouping even no blood group could be detected from the burned stained cloth debris. The blood stain positive samples after the detection were isolated by using QIAamp[at]Kit and 35µl volume of DNA was eluted. The DNA samples after isolation were observed by using 1% agarose gel, no visible bands were observed in gel electrophoresis unit. The isolated DNA from the sample was analyzed for the PCR using Global filer as per manual. The amplified PCR products allowed for the genotyping through AB 3500 genetic analyzer kit. The profiles in samples marked as S1, S2, S3, S4, S5 and S6 observed and they belong to separate human male/female individual where in sample S3 does not show complete profile which may due to highly degraded/ very minute amount of DNA recovered. The S4 consist single female individual.

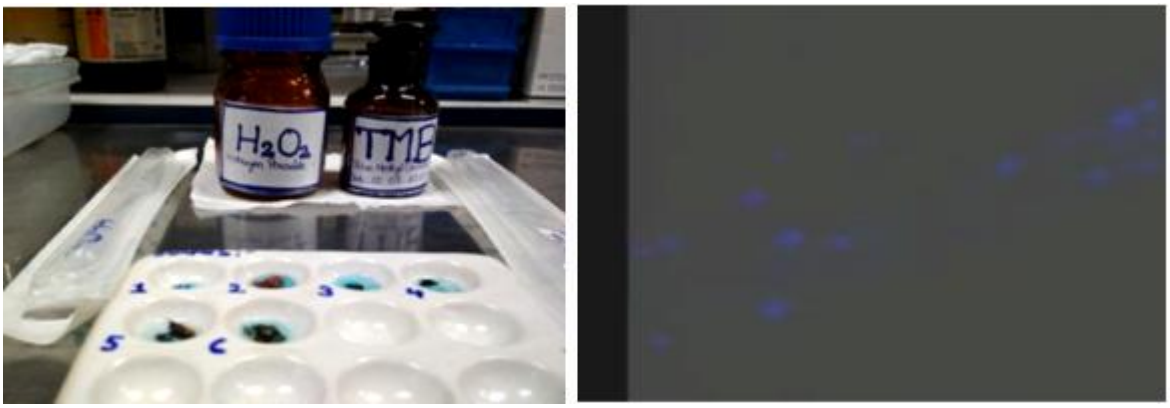

Figure 2: Detection of Blood TMB and Luminol spray reagent

Figure 3: Detection of Origin of Species

Table 2: Detection of Blood by TMB, Luminol, Origin of species and Blood grouping

S. No.	*Samples*	*Tetra Methyl Benzidine (TMB)*	*Luminol spray*	*Origin of Species*	*Blood group*
1.	S - 1	Positive	Positive	Not detected	Not detected
2.	S - 2	Positive	Positive	Not detected	Not detected
3.	S - 3	Faint Positive	Positive	Not detected	Not detected
4.	S - 4	Positive	Positive	Not detected	Not detected
5.	S - 5	Faint Positive	Positive	Not detected	Not detected
6.	S - 6	Faint Positive	Positive	Not detected	Not detected

*1, 2, 3, 4, 5 and 6 – S1, S2, S3, S4, S5 and S6 respectively
*H - Anti Human Sera
*C - Human Control

Table 3: PCR Cycle

Initial – Incubation Step	*29 Cycles*		*Final Extension*	*Final Step*
95^0C 1 min	94^0C 10 sec	59^0C 90 sec	60^0C 10 min	4^0C (∞)

Table 4: Allelic distribution of the sample

Marker	***S - 1***	***S - 2***	***S - 3***	***S - 4***	***S - 5***	***S - 6***
D3S1358	15, 16	16, 18	-	18	15, 16	15, 16
VwA	16, 17	15, 17	-	15, 19	16, 18	17, 18
DI6S539	10, 11	10, 12	-	12	9, 11	9, 10
CSF1PO	12	11	-	10, 12	12	10, 12
TPOX	11	9, 11	-	11	8, 11	8, 10
Y - indel	-	-	-	-	-	2
AMEL	XX	XX	XY	XX	XX	XY
D8S1179	11, 12	13	11, 15	14, 15	11, 12	12, 15
D21S11	28, 32.2	30, 33.2	30.2	28, 29	28, 32.2	28, 30
D18S51	14, 15	13, 14	-	12, 15	14, 15	14, 15
DYS391	-	-	-	-	-	-
D2S441	11	10, 11	11	10, 11	10, 11	11
D19S433	13, 14	13, 16.2	14	13, 15	13, 14	13
THO1	9	6, 7	8	9, 10	5.3, 9	9
FGA	21, 24	21	-	24	21, 24	24, 25
D22S1045	15, 17	11, 15	15	15, 16	15, 17	16, 17
D5S818	9, 12	11	11, 12	11, 12	12	9, 12
D13S317	8, 9	8	-	8, 12	8, 9	8, 9
D7S820	10, 11	11	11	8, 9	10, 11	8, 10
SE33	18, 28.2	19, 29.2	-	18, 30.2	18, 28.2	18
D10S1248	14, 16	13, 16	13	13, 14	14, 16	11, 13
D1S1656	13, 16	12, 16	-	13, 15	16, 19.3	14, 16
D12S391	23	17.3, 24	-	21, 22	23, 24	22, 23
D2S1338	17, 20	23, 24	-	21, 23	17, 25	23, 25

4. Summary and Conclusion

The study concludes that the blood was transferred in stain and then set on fire in controlled burn on open flame the help of flame to produce more realistic situation. Presumptive tests are effective for identification of bloodstain on burnt cloth debris. Luminol test is more effective in comparison to Tetra Methyl Benzidine for the detection of blood stain on burnt cloth debris. Luminol is more sensitive but its visibility is for few seconds and visible in total dark. All the samples after detection, origin and grouping were subjected to the DNA isolation. No origin and blood group had been developed from any of the sample though the genetic profile had been developed. The samples S1, S2, S4, S5 and S6 show genetic profile and it shows the male and female individual (s) respectively. Only S3 shown the partial profile which may due to degradation and/or very minute amount of DNA recovered or may because of cloth surfaces. However the amelogenin locus amplified successfully and indicate male individual. The research will further carried with more samples (Blood samples and different fabrics). The study concluded that luminal is effective than other presumptive test, the because it can cover a large surface area like the ashes which were difficult to collect it was sprayed over them and chemiluminescence produced which was very bright so it is convenient to analyze. The individualization from burned cloth debris is possible where the blood grouping and origin test were failed to do so.

5. Future Scope

The study will help uncover critical areas in the field of Forensic Science that many researches will be able to able to explore in the crime scene investigation as well as biotechnology. The study will help Crime Scene Investigators and Police to adopt wise tendency for collection and detection of evidences.

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Author Profile

Khalida Nazeer, completed masters in Forensic Science with 10 CGPA and working as Forensic Professional in Central Forensic Science Laboratory, Kolkata, West Bengal India for two years, also worked as Forensic Analyst in Infinivis Global Pvt. Ltd Allahabad. One research paper had been published in International Journal.

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