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Study of Protein in Dried Blood Stain with Respect to Time for Forensic Consideration

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***Abstract*-**Blood is an important body fluid as it is made up of various cell, protein, platelets, plasma, and other solutes. Blood constitutes about 8% of a person weight and it circulates near the surface of the skin. Almost all traumas to the body, therefore, results in the shedding of blood. Its red color makes it readily apparent at crime scence, and its residues are very difficult to remove completely. So, it has a very important entity in medico legal practices. The present study was carried out at Sam Higginbottom University and Technology Sciences and SRK pathology lab. In the present study unknown Blood samples were collected from the SRK pathology lab and chiffon clothes were also been used. The purpose of this study was for the identification of different type of protein present in the unknown blood sample for their individualization. The blood recovered at the scene of crime is generally in dried state. Blood is most frequently observed biological evidence at the crime scene pertaining to murder, sexual assault, and accidental cases. So it is very necessary to examine blood which assists the forensic analyst in an enhanced way.

***Keywords*-** Blood, Biological evidence, dried state, forensic science.

I. INTRODUCTION

Blood is a constantly circulating liquid providing the body with nutrition, oxygen, and waste elimination. Blood is mainly liquid, with abundant cells and proteins suspended in it a, making blood "thicker" than unadulterated water. The emblematic person has about 5 litres (more than a gallon) of blood .Liquid called plasma makes up about half of the satisfied of blood, which carry oxygen to the tissues. Proteins are important structure blocks of all cells and tissues. Proteins are essential for the bodies of growth, development, and health. Blood contains albumin and globulin. Albumin proteins remain fluid from leaking out of our blood vessels. Globulin proteins play an important role in our immune system. The detection of the character and quantity of specific sets of proteins in blood or other specimens Protein profiling may be used as a means of screening for cancer recurrence in previously treated patients or in patients with multiple risk factors for an illness. It may also aid in the crafting of therapies, e.g; by demonstrating by a particular disease is susceptible to a specific drug Since blood evidence associated with a crime can provide information that may solve the case, it is essential to correctly document, collect, and preserve this type of evidence. Improperly handled blood evidence can weaken or destroy a potential source of facts in a case. Properly collected and preserved blood evidence can establish a strong link between an individual and a criminal act. For this purpose the study for the identification of different type of protein present in the unknown blood sample for their individualization was carried out.

II. METHODOLOGY

Unknown blood sample were collected from SRK pathology lab and applied over the cloth (shiffon) and then left over for 5-10 minutes. Until it become completely dried. Thus, sample was prepared. Now, the sample which was prepared was extracted with distilled water. For the extraction process Petri dish was taken and then dried blood stain sample was placed inside the Petri dish. After, this distilled water was poured inside the Petri dish. Now with the help of twizer the stain was extracted. The extracted sample was then taken into the test tube and sample number labeling was done on the particular test tube. Blank,

standard, and unknown was labeled on the different test tube. These 3 different labeled test tubes were examined with 3 different procedures. At last estimation was noted down.

(FOR SAMPLE KEPT AT 37^0 C) The five test tubes was taken and marked as standard. 1ml protein reagent was added. Followed by 20 µl standard (total protein standardization) was added in each test tube. The solution was mixed well by shaking it thoroughly.). Now test tube was incubated for 5 – 10 minutes at 37^0 C inside incubator. After the incubation it was run on semiautoanalyzer. Estimation was noted down. The process was repeated after 15 days.

(FOR SAMPLE KEPT AT ROOM TEMPERATURE) The five test tube was taken and marked as standard. 1ml protein reagent was added. Followed by 20 µl standard (total protein standardization) was added in each test tube. The solution was mixed well by shaking it thoroughly.). Now test tube was incubated for 5 – 10 minutes at 37^0 C inside incubator. After the incubation it was run on semiautoanalyzer. Estimation was noted down. The process was repeated after 15 days. This process was repeated after 15 days.

Test for protein (Test: Unknown Sample) A test tube was labeled as a test. 1ml protein reagent was added. Followed by the solution 20 µl unknown samples were added in the test tube. Now test tube was incubated for 5 – 10 minutes at 37^0 C inside incubator. After the incubation it was run on semiautoanalyzer. Estimation was noted down. Similarly, the whole processes were applied for albumin. After completion of this procedure than estimation and ratio of globulin was find out.

III. RESULTS AND DISSCUSION

In this study I observed five sample for protein profiling there was different proteins were identify in dried blood stain ,also some variations in protein profile for individualization and variation in proteins concentration with respect to time

Table 4.1 Estimation of protein in dried blood stain on semiautoanalyzer

S.no.	Samples	Extraction (room temperature)	Incubator (37^0 C)	Freeze (2-8 ^{o} C)
1	A	0.59gm/dl	0.31gm/dl	5.96gm/dl
2	B	0.57gm/dl	0.43gm/dl	6.00gm/dl
3	C	0.31gm/dl	0.32gm/dl	7.80gm/dl
4	D	0.55gm/dl	0.27gm/dl	6.91gm/dl
5	E	1.21gm/dl	0.55gm/dl	7.91gm/dl

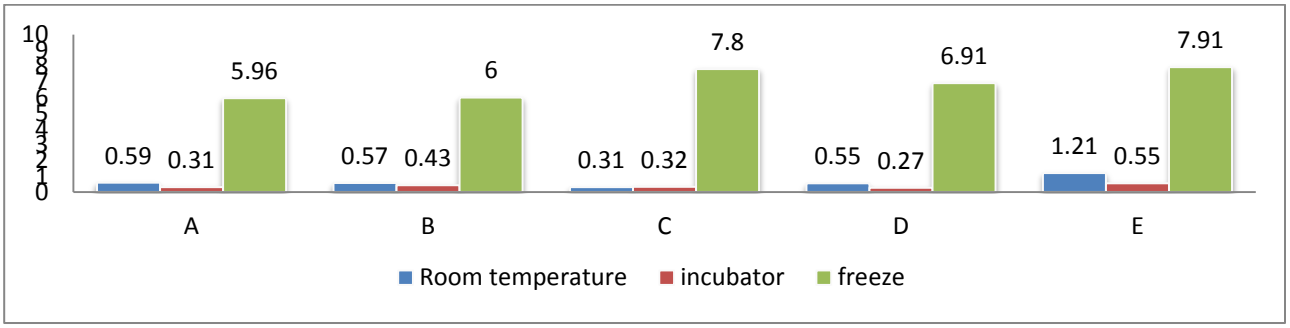

Fig 4.1 Graph of estimation of protein in dried blood stain at time interval

Table 4.1 shows the estimations of protein in dried blood stain on semiautoanalyzer After extraction of blood samples from dried cloth at different temperature that is room temperature, incubating at 37^0 C , and freeze at 2-8^{0}C.

Table 4.2 Estimation of protein in dried blood stain at time interval of 30days.

S.no.	Samples	Extraction (room temperature)	Incubation 37^0 C	Freeze (2-8 ^{o} C)
1	A	0.86 gm./dl	0.15gm/dl	5.97 gm./dl
2	B	1.00 gm./dl	0.28 gm./dl	5.76 gm./dl
3	C	1.68 gm./dl	0.17 gm./dl	6.24 gm./dl
4	D	1.01 gm./dl	0.18 gm./dl	6.17 gm./dl
5	E	1.20 gm./dl	0.58 gm./dl	6.31gm/dl

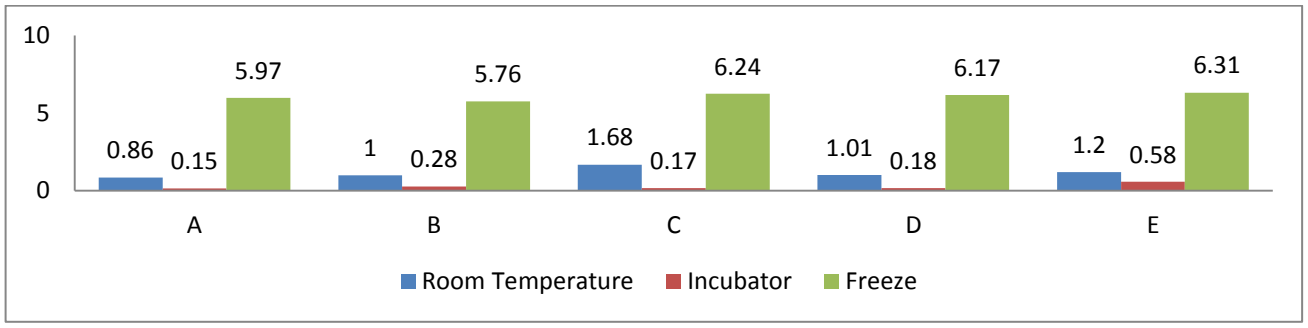

Fig 4.2 Graph of estimation of protein in dried blood stain at time interval of 30 days

Table 4.2 shows the estimations of protein in dried blood stain on semiautoanalyzer After extraction of blood samples from dried cloth at different temperature that is room temperature, incubating at 37^0 C , and freeze at 2-8^0C.this was conducted at time interval of 30 days.

Table 4.3 estimation of protein in dried blood stain at time interval of 60days

S.N0.	Samples	Extraction room temperature	Incubator (37 ^{0} C)	Freeze (2-8 ^{o} C)
1	A	0.31gm/dl	0.02gm/dl	4.89gm/dl
2	B	0.36gm/dl	0.08gm/dl	4.76gm/dl
3	C	0.29gm/dl	0.03gm/dl	4.48gm/dl
4	D	0.24gm/dl	0.06gm/dl	4.27gm/dl
5	E	0.86gm/dl	0.19gm/dl	4.59gm/dl

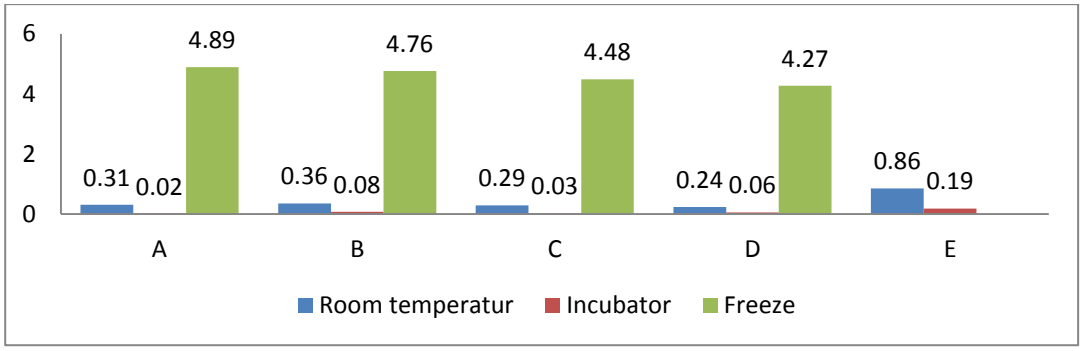

Fig 4.3 Graph of estimation of protein in dried blood stain at time interval of 60 days

Table 4.3 shows the estimations of protein in dried blood stain on semiautoanalyzer After extraction of blood samples from dried cloth at different temperature that is room temperature, incubating at 37^0 C , and freeze at 2-8^0C.This was conducted at time interval of 60 days.

Table 4.4 Estimation of Albumin in dried blood stain on semiautoanalyzer

S.NO.	**Samples**	**Extraction (room temperature)**	**Incubator(37 ^{0} C)**	**Freeze**
1	A	0.20gm/dl	0.22gm/dl	4.44gm/dl
2	B	0.25gm/dl	0.16gm/dl	4.05gm/dl
3	C	0.10gm/dl	0.16gm/dl	4.49gm/dl
4	D	0.24gm/dl	0.22gm/dl	4.82gm/dl
5	E	0.71gm/dl	0.42gm/dl	5.17gm/dl

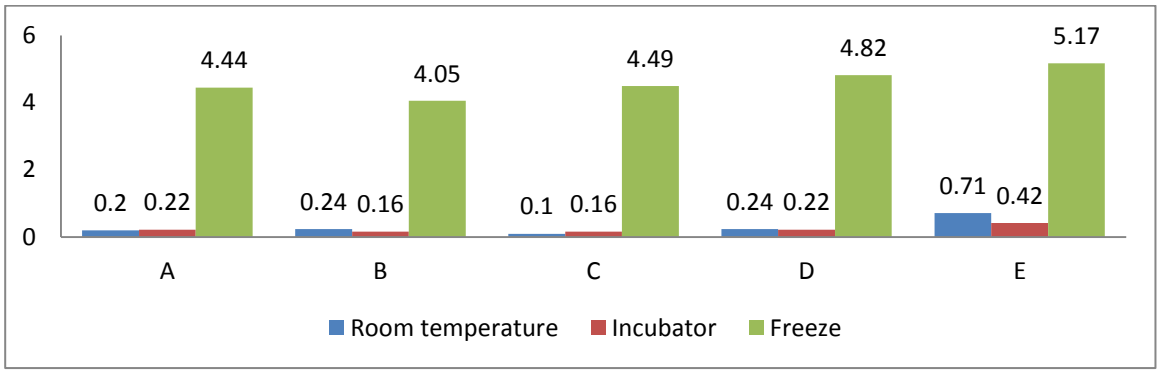

Fig .4.4 Graph of estimation of Albumin in dried blood stain on semiautoanalyzer

Table 4.4 shows the estimations of albumin in dried blood stain on semiautoanalyzer After extraction of blood samples from dried cloth at different temperature that is room temperature, incubating at 37^0 C , and freeze at 2-8^0C.

Table 4.5 estimation of Albumin in dried blood stain at time interval of 30days

S.NO.	Samples	Extraction (room temperature)	Incubator (37 ^{0} C)	Freeze (2- 8 ^{o} C)
1	A	0.15gm/dl	0.25gm/dl	3.87gm/dl
2	B	0.19gm/dl	0.22gm/dl	3.60gm/dl
3	C	0.17gm/dl	0.23gm/dl	3.72gm/dl
4	D	0.18gm/dl	0.29gm/dl	3.81gm/dl

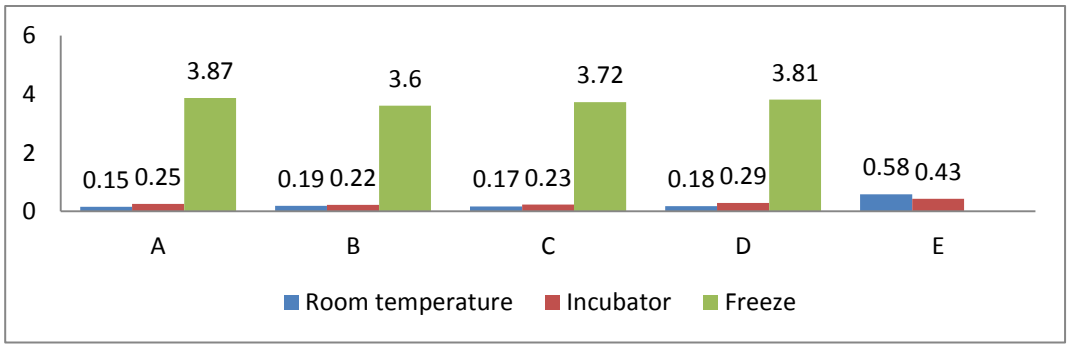

Fig 4.5 Graph of estimation of albumin in dried blood stain at time interval of 30 days

Table 4.5 shows the estimations of albumin in dried blood stain on semiautoanalyzer After extraction of blood samples from dried cloth at different temperature that is room temperature, incubating at 37^0 C , and freeze at 2-8^0C.this was conducted at time interval of 30 days.

Table 4.6. Estimation of total Albumin in dried blood stain at time interval of 60 days

Sr. no	**Samples**	**Extraction (room temperature)**	**Incubator 37 ^{0} C**	**Freeze(2-8 ^{O} C)**
1	A	0.02gm/dl	0.10gm/dl	2.92gm/dl
2	B	0.04gm/dl	0.08gm/dl	2.08gm/dl
3	C	0.03gm/dl	0.07gm/dl	2.21gm/dl
4	D	0.06gm/dl	0.09gm/dl	2.16gm/dl
5	E	0.13gm/dl	0.16gm/dl	3.00gm/dl

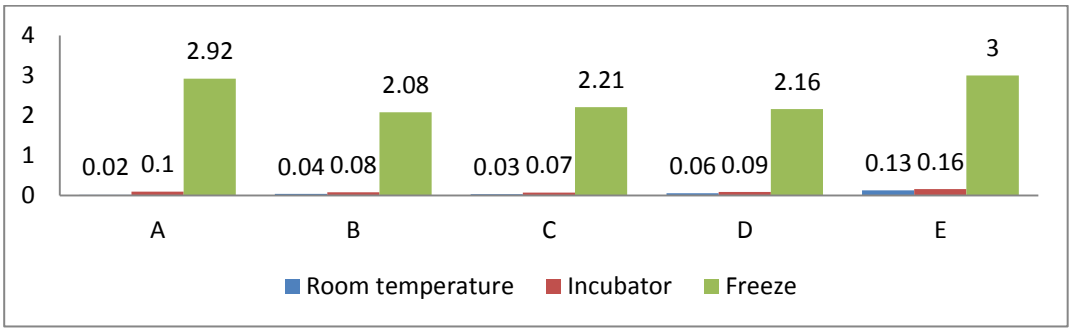

Fig.4.6 Graph of estimation of albumin in dried blood stain at time interval of 60 days

Table 4.6 shows the estimations of albumin in dried blood stain on semiautoanalyzer After extraction of blood samples from dried cloth at different temperature that is room temperature, incubating at 37^0 C , and freeze at 2-8^{0}C.This was conducted at time interval of 60 days.

Table 4.7 Estimation of globulin in dried blood stain on semiautoanalyzer

S.no	Samples	Extraction room temperature	Incubator 37 ^{O} C	Freeze (2-8 ^{O} C)
1	A	0.39gm/dl	0.09gm/dl	1.52gm/dl
2	B	0.32gm/dl	0.27gm/dl	1.95gm/dl
3	C	0.21gm/dl	0.16gm/dl	3.31gm/dl
4	D	0.31gm/dl	0.05gm/dl	2.09gm/dl
5	E	0.5gm/dl	0.13gm/dl	2.74gm/dl

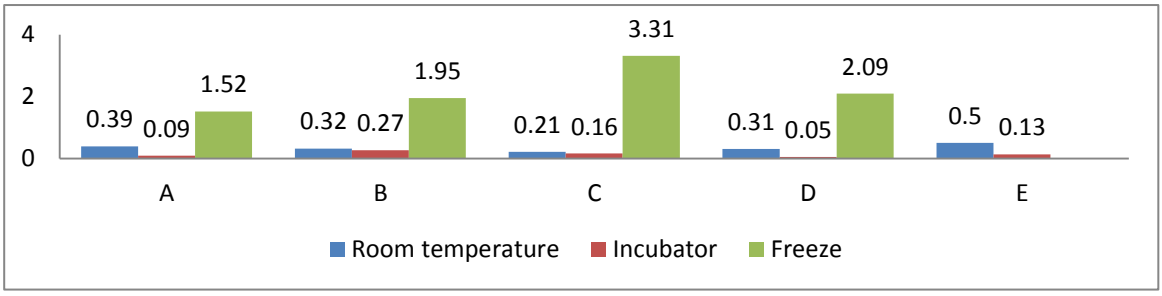

Fig4.7 Graph of estimation of globulin in dried blood stains on semiautoanalyzer

Table 4.7 shows the estimations of globulin in dried blood stain on semiautoanalyzer After extraction of blood samples from dried cloth at different temperature that is room temperature, incubating at 37^0 C , and freeze at 2-8^{0}C.

Table 4.8 Estimation of Globulin in dried blood stain at time interval of 30days

S.no	Sample	Room Temperature	Incubator(37 ^{O} C)	Freeze (2-8 ^{O}C)
1	A	0.71gm/dl	0.36gm/dl	2.1gm/dl
2	B	0.81gm/dl	0.62gm/dl	2.16gm/dl
3	C	1.51gm/dl	0.01gm/dl	2.52gm/dl
4	D	0.83gm/dl	0.02gm/dl	2.36gm/dl
5	E	0.62gm/dl	0.27gm/dl	2.25gm/dl

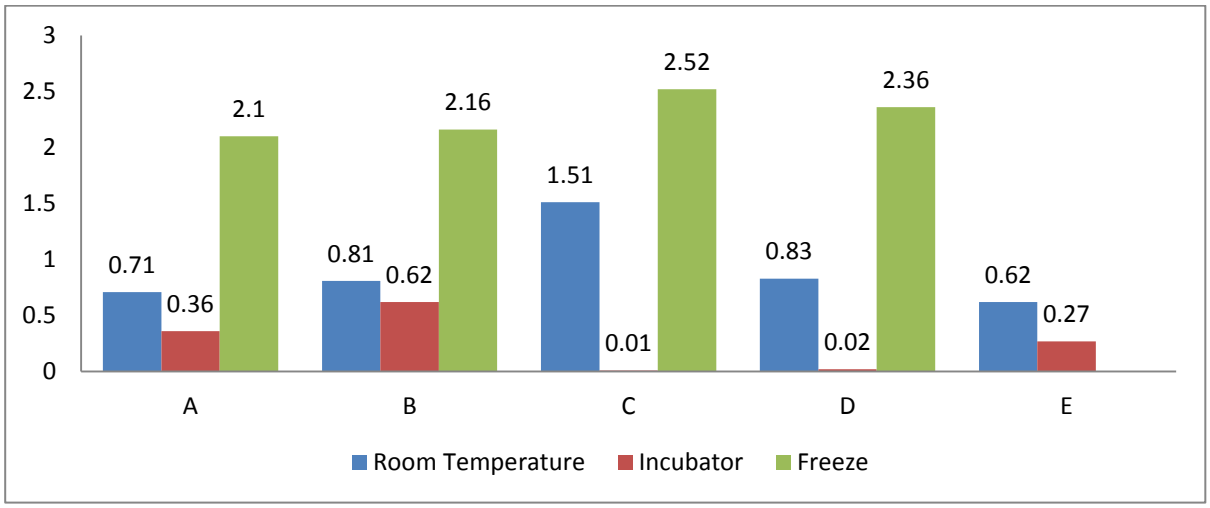

Fig 4.8 Graph of estimation of globulin in dried blood stain at time interval of 30 days

Table 4.5 shows the estimations of globulin in dried blood stain on semiautoanalyzer After extraction of blood samples from dried cloth at different temperature that is room temperature, incubating at 37^0 C , and freeze at 2-8^0C.this was conducted at time interval of 30 days.

Table 4.9 Estimation of Globulin in dried blood stain at time interval of 60 days

S.no	Samples	Room Temperature (25^0 C)	Incubator (37^0 C)	Freeze (2^0-8^0 C)
1	A	0.29gm/dl	0.1gm/dl	1.97gm/dl
2	B	0.32gm/dl	0.72gm/dl	2.68gm/dl
3	C	0.26gm/dl	0.23gm/dl	2.27gm/dl
4	D	0.18gm/dl	0.51gm/dl	2.11gm/dl
5	E	0.73gm/dl	0.03gm/dl	1.59gm/dl

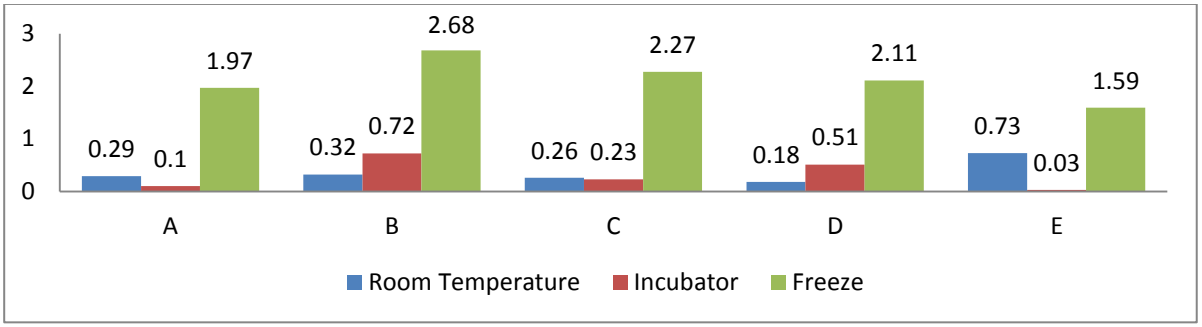

Fig 4.9 Graph of estimation of globulin in dried blood stain at time interval of 60 days

Table 4.6 shows the estimations of globulin in dried blood stain on semiautoanalyzer After extraction of blood samples from dried cloth at different temperature that is room temperature, incubating at 37^0 C , and freeze at 2-8^0C.This was conducted at time interval of 60 days.

IV. DISCUSSIONS

The present study was aimed for the identification of different protein present in different dried bloodstains, there variations in profile and variations in the concentration according to time .For this purpose the samples were collected from SRK pathology lab Phulpur, Allahabad. After the collection of sample then it was extracted. After extraction the sample was kept at different temperatures ie; Room temperature, Incubator and freeze. At last sample was run in the semiautoanalyzer and estimation was noted down. The complete work was done for 3 months and different variation at different temperatures was found. Similar

type of work was conducted by the Kulak et al; 2014 for protein profiling. Using semiautoanalyzer, he studied the proteomics typically employs multistep sample preparation workflows that as subject to sample contamination and loss

V. CONCLUSIONS

The present study entitled "Study of proteins in dried blood stain with respect to time for forensic consideration was carried out at the department of forensic Science, Allahabad, as per objectives it was concluded that: Different proteins present in dried blood stain i.e.; albumin and globulin, total protein were successfully identified up to 60 days Profiling of the identified proteins in blood of different individuals was prepared and significant variations were observed among the protein profile of suspected blood and stain may sometime help in identification of a person .It was concluded that different variations in protein concentration were found with respect to time

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