



ABO blood stain typing by forwarding and reversing method of absorption-elution technique with same fiber/cloth piece

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ABSTRACT

Blood group typing is being considered vital and has always been the primary procedure to be followed in the study of forensic serology. Using this method the same fiber/cloth piece can be used several times to obtain the similar results. This technique now reduces the time cost and effort that was put into it earlier. With "ABO blood stain typing by Forwarding and Reversing Method of Absorption-Elution Technique" different concentrations of blood, packed cells obtained from different people and different batches of anti-sera to can be used to draw to concrete results. Blood stained cloth/fiber samples obtained from 18 different cases were used for ABO typing and the procedure was repeated 7-10 times for same sample with Forwarding and Reversing Absorption-Elution technique. All cloth/fiber samples yielded the consistent results even after several repeated trials.

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Introduction

The ABO blood group system is widely used in Serological tests. While several techniques have been developed which detect ABH antigens (Ladd C. et.al, 1996). problems kept arising when the analyzed samples were seriously degraded (Zachová M. et. al, 2004). "Absorption Elution Technique" is being used for stains received in cases like rape, attempted murder, murder etc.

It is challenging to the Forensic Serologist to type blood grouping of a blood stain while it is faint, since the quantity received is far less, for example blood stains found with accused, where we need to repeat the analysis many times before conclusion of group of the stain. The determination of the ABO grouping from the body often encounters difficulty due to hemolytic erythrocytes, and putrefaction, mummification or skeletonization of the body during post-mortem interval (Nishi K., 2005).

ABO blood typing is very old method to know the blood group of a person. An indirect method to know the ABO blood group is the "Absorption-Elution Technique" where typing of blood group is done on cellulose acetate sheet, and after the analysis note the results and discard the cellulose sheet. Absorption-inhibition procedures have been used ever since 1920s, for determining the ABH antigens of dried blood (Schütze, H., 1921), although the Absorption-Elution technique originally devised by Siracusa (Siracusa. V., 1923) and refined by Kind (Kind. S. S., 1960) is now employed almost exclusively for this purpose in most laboratories (Gaensslen R. E., 1983; Lee H. C.; 1982).

The VDRL (Venereal Disease Research Laboratory) slide facilitates the Scientist to repeat the analysis many number of times, saving time in the process of sticking the samples again and again and also reducing the cost of it.

However more advanced techniques like DNA testing are employed in potentially important and confusing cases, in

developing countries like India it is not always possible to perform a costly process like DNA testing on all the samples. A serological technique like ABO blood typing therefore is frequently used by forensic scientists to help the law enforcement department to draw primary idea from the scene of crime (SOC). Merits and demerits of different ABO blood grouping methods like red cell agglutination, absorption elution, histochemical method and DNA genotyping have been highlighted (Nishi K., et.al., 2005). In the present study, same cloth/fiber piece of forensic origin has been used three times to yield successful results for blood group typing. By this technique different concentration of blood, packed cells obtained from different persons and also tried different batches of anti-sera by using same piece of cloth.

Material And Method:

Samples:

Forensic samples were used for the study that comprised of fiber/cloth pieces soaked in blood of different people. The samples were collected during a period of one to two months old which were preserved in Indian conditions at temp of 28 to 40°C.

Decontamination of samples:

To remove the contamination of bacterial and fungal origin stains were heated at 100°C for one hour.

Blood Grouping:

The procedures were carried on VDRL slide by sticking 1.5x0.3 cm² fiber in each cavity of the slide (POLAR INDUSTRIAL CORPORATION, Mumbai, India) (Fig1). Anti-sera of monoclonal antibodies of A, B, and H were added which are commercially available (POLAR INDUSTRIAL CORPORATION, Mumbai, India) (Fig2). Followed the standard Absorption-Elution Method and noted the results (Fig3).

The slides were then washed with 56°C warm saline thoroughly and removed whole of the saline and added one drop

of fresh saline in each cavity of VDRL slide. Later the slides were incubated at 56°C for 40 to 45 minutes and again washed the slide 56°C warm saline 2-3 times. Finally the slides were air dried and Absorption -Elution method was repeated again. The blood grouping procedure was repeated by the "Forwarding and Reversing of Absorption-Elution Method of Blood stains typing with same fiber/cloth piece for 7-10 times to yield concordant results.

Care was taken to make sure that color of stains of selected cloth piece was same. If stain was faint then width of the fabric was increased to obtain uniform amount of antigens in all cavities. A positive controls of known blood groups and blank sample as negative were also run along with the samples.

Results and discussion:

Sixty seven (67) samples obtained from 18 different cases (Table1) were used for ABO typing and the results given by fiber/cloth samples were matched with other samples of same origin that are available. Most of them are attempted murder cases where reference blood group is obtained from victims and compared. In murder cases also samples from scene of crime (SOC), accused cloths or weapons recovered from accused or SOC and deceased cloths yielded same blood group connecting accused with SOC and deceased cloth samples like inner vest, pant, shirt, cap, sari, etc. except in case number item 3 i.e blood stains on short which is recovered from scene of crime is B blood group where item of deceased i.e shirt and inner vest were showing O blood group. Moreover, the fiber/cloth samples were tested repeated three times using the reversing technique and gave same results. Three times packed cell of concentration 1.25% , 1.5% and 1.75% respectively were added, as same concentration of the antigens for analysis is taken (By taking same size of cloth pieces/fiber and color of the stains is same). Three times the times results were consistent.



Fig1: Samples without anti-sera



Fig2: Samples with anti-sera



Fig 3: Samples after Elution

Conclusion:

Accuracy of ABO typing is very important for forensic analysis. However small sample size or decayed or disintegrated samples and possible contaminations of cloth samples make it difficult to be tested multiple times if required during the course of law.

The Forwarding and Reversing Technique of Absorption-Elution Method makes it possible for same sample to be tested again and again with accuracy without any requirement of fresh samples each time. This technique can be useful for forensic scientists struggling with improper supply of blood soaked samples to the laboratory.

In standard procedure we found that cotton fabric yielded good results when compared to synthetic materials. Although the samples used for ABO typing can not be further used for DNA typing, the method is extremely meritorious as it uses minimal amount of sample and the remaining sample stays intact and undisturbed for further studies like DNA profiling.

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Reference:

- Ladd, C., Bourke, M. T., Scherezinger, C. A., Pagliaro, E. M., Gaensslen, R. E., and Lee, H. C., (1996). A PCR-Based Strategy for ABO Genotype Determination. *Journal of Forensic Sciences, JFSCA*, Vol. 41, No. 1: 134-137.
- Zachová M, Zelený M, Pexa T, Mazura I, Hirt M. (2004). Determination of ABO blood group system from degraded blood stains on serological and molecular genetic level *Soud Lek.* 49(3):43-6.
- Nishi K. (2005). ABO blood group typing in forensic autopsies. *Nihon Hoigaku Zasshi.* 59(2):111-7.
- Schtitze, H. (1921). Haemagglutination and its Medico-legal Bearing, with Observations upon the Theory of Isoagglutinins. *British Journal of Experimental Pathology.* Vol. 2: 26-33.
- Siracusa. V. (1923). La Sostanza Isoagglutabile del Sangue e la sua Dimostrazione per la Diagnosi Individuale delle Macchie. *Archivio di Antropologia Criminale. Psichiatria e Medichza Legale.* Vol. 43 (Vol. 14, 4th Series): 362-384.
- Kind, S. S. (1960). Absorption Elution Grouping of Dried Bloodstains on Fabrics. *Nature.* Vol. 187: 789-790.
- Lee. H. C. (1982). Identification and Grouping of Bloodstains. *Forens & Science Handbook.* R. Saferstein, Ed., Prentice Hall, Englewood Cliffs, NJ: 267-337.

Table 1: Result of blood grouping of samples obtained from different type of cases

	A	B	O	Group
Case No. 1				
1. Deceased Shirt	-	+	-	B
2. Deceased inner vest	-	+	+	B
3. Accused Shirt(small spots)	-	+	+	B
4. Pant(small spots)	-	+	+	B
Case No. 2				
1. Deceased Shirt	-	+	+	B
2. Deceased inner vest	-	+	-	B
3. Deceased monkey cap	-	+	-	B
4. Deceased Pant	-	+	-	B
Case No. 3				
1. Deceased Shirt	-	-	+	O
2. Deceased inner vest	-	-	+	O
3. Short (from SOC)	-	+	-	B
Case No. 4				
1. Deceased Shirt	+	+	+	AB
2. Deceased inner vest	+	+	-	AB
3. Accused shirt	+	+	+	AB
4. Accused T-shirt	+	+	-	AB
Case No. 5				
1. Deceased Shirt	-	+	+	B
2. Deceased inner vest	-	+	+	B
3. Knife(from accused)	-	+	+	B
Case No. 6				
1. Deceased Blouse	-	-	+	O
2. Deceased Saree	-	-	+	O
3. Accused Shirt	-	+	-	B
Case No. 7				
1. Deceased Shirt	+	-	+	A
2. Deceased lungi	+	-	-	A
3. Deceased Under wear	+	-	-	A
4. Knife (recovered from SOC)	+	-	+	A
Case No. 8				
1. Deceased Shirt	-	+	-	B
2. Knife(A1)	-	+	-	B
3. Metal rod(A2)	-	+	+	B
4. Metal rod(A3)	-	+	-	B
Case No. 9				
1. Bed sheet(SOC)	-	-	+	O
2. Deceased Sari	-	-	+	O
3. Deceased blouse	-	-	+	O
Case No. 10				
1. Deceased Shirt	+	-	-	A
2. Deceased inner vest	+	-	-	A
3. Stick (SOC)	+	-	-	A

Case No. 11					
1.	Deceased Shirt	+	+	-	AB
2.	Pillow cover	+	+	+	AB
Case No. 12					
1.	D1 Shirt	-	-	+	O
2.	D1 inner vest	-	-	+	O
3.	D2 Pant	-	-	+	O
4.	D2 inner vest	-	-	+	O
5.	D 3 shirt	-	-	+	O
6.	D3 Pant	-	-	+	O
7.	Broken bottle	-	-	+	O
Case No. 13					
1.	Deceased Kurtha(top)	+	-	-	A
2.	Deceased Pajama(bottom)	+	-	-	A
3.	Deceased Chunni	+	-	-	A
Case No. 14					
1.	Deceased Towel	+	-	+	A
2.	Deceased inner vest	+	-	-	A
3.	Accused Sari	+	-	+	A
4.	Axe	+	-	-	A
Case No.15					
1.	Cement floor piece(SOC)	+	+	-	AB
2.	Wooden block(SOC)	+	+	+	AB
3.	Wooden stick(from accused)	+	+	+	AB
4.	Deceased Pant	+	+	-	AB
5.	Deceased shirt	+	+	+	AB
6.	Deceased PM Blood	+	+	-	AB
Case No.16					
1.	Stone(SOC)	-	+	-	B
2.	Stone(SOC)	-	+	-	B
3.	Deceased inner vest	-	+	-	B
Case No. 17					
1.	Deceased Pant	-	+	+	B
2.	Deceased shirt	-	+	-	B
3.	Knife(from accused)	-	+	+	B
4.	Deceased blood(Post mortem)	-	+	+	B
Case No. 18					
1.	Axe(recovered from accused)	-	+	-	B
2.	Deceased Inner vest	-	+	-	B
3.	Deceased Shirt	-	+	+	B

● Nishi K., Rand S., Nakagawa T. et.al.. ABO Blood Typing from Forensic Materials - Merits and demerits of detection methods utilized in our laboratories, and biological significance of the antigens. Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology, 2005; Vol. 6, No. 2 (July - December

2005)

http://www.anilaggrawal.com/ij/vol_006_no_002/papers/paper001.html; Published July 1, 2005, (Accessed: February 05, 2013)