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Typing Rh factor of Bloodstains

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Abstract *- The Rh blood group system contains various blood-group antigens, but few are well known in clinical serology. The Rh factor i.e. Rh positive and Rh negative depends on the presence and absence of the D-antigen on erythrocytes. In criminal cases like murder, attempted murder and sexual assault cases, it is necessary to know the blood group of an unknown blood sample found at the crime scene and its degree of association with the deceased or victim, the suspect and the crime or crime scene. Typing of Rh factor in bloodstains is uncommon in forensic samples due to difficulties in typing. The present study proved that the Mixed Agglutination Technique is a suitable technique to type Rh factor of bloodstains.*

Keywords *- Anti-D antigen, Bloodstains, Forensic samples, Mixed Agglutination, Rh factor typing.*

I. INTRODUCTION

The Rh blood group is one of the most complex human blood group, first discovered in Rhesus monkey. It was discovered in early 19th century and this is the second important blood group after ABO blood group system in the field of transfusion medicine. The major application of the Rh system for human is the danger of RhD incompatibility between mother and fetus. The Rh factor i.e. Rh positive and Rh negative depends on the presence and absence of the D-antigen on erythrocytes. In criminal cases like murder, attempted murder and sexual assault, it is crucial to know the blood group of unknown blood sample found at the crime scene and also the person to whom the blood belongs to and how he or she is connected to the crime or even the crime scene.

It is uncommon to type Rh factor of bloodstains in forensic applications due to primary difficulties while typing bloodstains and problems kept arising especially when samples were seriously degraded [1]. Since Rh antibodies are IgG they bind best at 37^0C and their reactions will be observed with the indirect antiglobulin technique [2]. The Rh antigen is Ig, therefore, there are fewer antigen binding sites than ABO. It is well known that the most commonly found Rh antibodies do not agglutinate when Rh positive erythrocytes are suspended in simple salt solutions, to obtain direct agglutination with these so-called "incomplete" antibodies [3]. An indirect technique that is commonly used in forensic science laboratories to type ABO blood grouping and is called "Mixed Agglutination Method". The technique is based on the principle that the cells are specifically bound to antibody molecules on incubation in which the other binding sites have specifically bound to the bloodstains antigen in the absorption stage. In a positive test, the cells line up as beads along the length of the bloodstained fiber [4]. The same technique was employed to type Rh factor of forensic bloodstains in the present study.

II. MATERIALS AND METHOD

A. Collection of samples

Known ABO blood group bloodstain samples were taken to study. These samples were of murder and attempt murder cases, which were collected between 10 days to one month.

B. Decontamination of samples

To remove the contamination of bacterial and fungal origin, stains were heated at 100°C for one hour (5).

C. Preparation of packed cells

Rh positive and Rh negative blood samples were collected from local hospitals and cells were washed three times with chilled saline by centrifugation to remove serum and thus collected and packed cells were taken for analysis.

D. Blood Grouping

The procedure was carried on the VDRL slide by taking 2 bloodstains fibers of 1 cm long in two separate cavities of the slide (POLAR INDUSTRIAL CORPORATION, Mumbai, India) and marked as Rh + and Rh-. Anti-sera of monoclonal antibodies of Anti-D were added in both cavities that are commercially available

(POLAR INDUSTRIAL CORPORATION, Mumbai, India). The fibers in both cavities were teased and separated with needles and kept at 4^0C for 2 hours for absorption. After 2 hours, excess anti-sera was removed without disturbing the fiber with a filter paper, tossed a drop of saline in it and again removed saline slowly from cavities. In the similar manner, fibers were washed three times to remove excess antisera. After removing antisera, known packed cells of Rh positive and negative of 0.5% blood cells were added in two cavities respectively. Later, they were kept at 4^0C for 2 hours. After 2 hours slides were observed under microscope for agglutination on the fiber. The Rh positive and Rh negative control bloodstain samples were typed along with the samples (Fig.1).

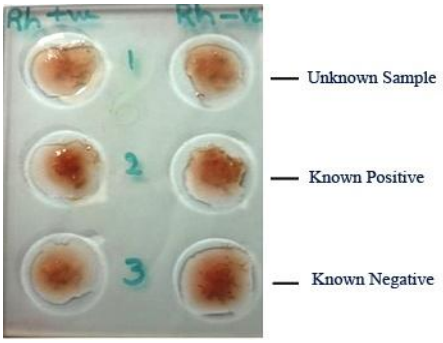

Fig.1. Samples with antisera

III. RESULTS AND DISCUSSION

The known Rh+ bloodstain sample showed a beaded agglutination on the fiber in the Rh+ cavity and no agglutination was present in the Rh- cavity. Known Rh- bloodstain sample showed no agglutination in both cavities. Except two samples of case number 12, i.e. third deceased clothing, all case samples bloodstains showed agglutination in the Rh+ cavity and no agglutination was present in the Rh- cavity (Fig.2). In case number 12, bloodstains on third deceased clothing showed negative in both cavities.

In the first step, Anti-D sera was added in both the cavities that were marked as Rh positive and Rh negative to allow to form a complex between antigen and antibody. In the second step, Rh positive blood cells were added in the cavities that were marked as Rh+ and Rh - blood cells in the cavities that were marked as Rh- to prove that the bloodstains is not of Rh positive blood. (In the second step, samples that were marked Rh- due to lack of suitable antigen a visible complex will not be formed).

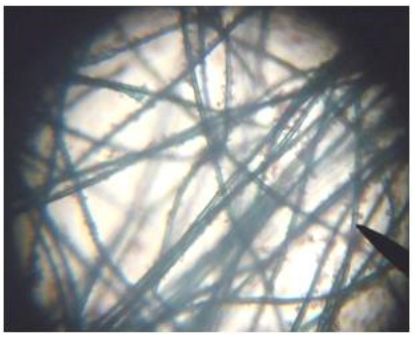

Fig.2. Sample with agglutination in Rh positive cavity

Table 1

	A	**B**	**O**	**Group**	**Rh** + or -
Case No. 1					
Deceased Shirt	-	+	-	B	+
Deceased inner vest	-	+	+	B	+
Accused Shirt (small spots)	-	+	+	B	+
Pant(small spots)	-	+	+	B	+
Case No. 2					
Deceased Shirt	-	+	+	B	+

Deceased inner vest	-	+	-	B	+
Deceased monkey cap	-	+	-	B	+
Deceased Pant	-	+	-	B	+
Case No. 3					
Deceased Shirt	-	-	+	O	+
Deceased inner vest	-	-	+	O	+
Short (from SOC)	-	+	-	B	+
Case No. 4					
Deceased Shirt	+	+	+	AB	+
Deceased inner vest	+	+	-	AB	+
Accused shirt	+	+	+	AB	+
Accused T-shirt	+	+	-	AB	+
Case No. 5					
Deceased Shirt	-	+	+	B	+
Deceased inner vest	-	+	+	B	+
Knife (from accused)	-	+	+	B	+
Case No. 6					
Deceased Blouse	-	-	+	O	+
Deceased Saree	-	-	+	O	+
Suspect Shirt	-	+	-	B	+
Case No. 7					
Deceased Shirt	+	-	+	A	+
Deceased lungi	+	-	-	A	+
Deceased Under wear	+	-	-	A	+
Knife (recovered from SOC)	+	-	+	A	+
Case No. 8					
Deceased Shirt	-	+	-	B	+
Knife (A1)	-	+	-	B	+
Metal rod (A2)	-	+	+	B	+
Metal rod (A3)	-	+	-	B	+
Case No. 9					
Bed sheet (SOC)	-	-	+	O	+
Deceased Sari	-	-	+	O	+
Deceased blouse	-	-	+	O	+
Case No. 10					
Deceased Shirt	+	-	-	A	+
Deceased inner vest	+	-	-	A	+
Stick (SOC)	+	-	-	A	+
Case No. 11					
Deceased Shirt	+	+	-	AB	+
Pillow cover	+	+	+	AB	+
Case No. 12					
D1 Shirt	-	-	+	O	+
D1 inner vest	-	-	+	O	+
D2 Pant	-	-	+	O	+
D2 inner vest	-	-	+	O	+
D 3 shirt	-	-	+	O	-
D3 Pant	-	-	+	O	-
Broken bottle	-	-	+	O	+

IV. CONCLUSION

The present study proved that the Mixed Agglutination Technique is a technique can be adapted to type Rh factor of forensic bloodstain samples. However, 85% of the population are Rh positive and whereas only 15% is of Rh negative, but it plays its own role in the individualization of the bloodstains.

In this process cotton fabric yielded good results when compared to synthetic materials. In typing of bloodstains by mixed agglutination technique a very less quantity is sufficient, so that the remaining sample can be used for further analysis like DNA typing.

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