PRELIMINARY INVESTIGATIONS INTO THE DETECTION OF THE ANTIGENS IN GROUP AB BLOOD AND SALIVA STAINS

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Introduction

The loss or decrease of A or B antigen from group AB blood and saliva stains has been occasionally observed in forensic case work. This study was undertaken to provide information on the frequency of this occurring.

The blood stains used in this study fell into two categories.

1) Stains varying in age from one to eight years. All these stains had been stored at room temperature.

2) Stains stored dry or moist at various temperatures and grouped at intervals over a period of two and a half months.

The saliva stains used in this study were stored at room temperature and grouped at intervals over six months.

Materials and Methods

The samples used in this study were liquid blood or salivas that had been sent to the laboratory in connection with criminal cases. The blood cells had been typed in the ABO system at the time of receipt, and A_1 and A_2 subtyping performed on some.

The stains that were over one year old had been made on cotton cloth and stored dry at room temperature and grouped by absorption elution.

In the study involving various conditions of storage five group AB blood samples were used. Ten stains were made from each sample. Five of these were allowed to dry overnight at room temperature and the other five were stored individually in polythene bags which were then enclosed in air-tight polystyrene boxes and stored in a moist condition.

A dry and moist stain was stored at each of the following temperatures: -20 C, 4 C, room temperature, 37 C and 52 C.

Therefore there were ten conditions of storage for the stains made from each blood sample.

For comparative purposes three group O blood samples were similarly made into stains and stored.

All the stains were grouped at intervals, starting when they were one day old and then after two, six and ten weeks.

Results

The results of typing group AB blood stains of one and two, five to six, and seven to eight years are given in table 1.

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Table 1
Grouping results from blood stains stored from 1 to 8 years at room temperature.

Age in Years	Type	Total No.	No. incorrectly grouped as A	or	B
1	AB	40	0		1
2	A_1B	8	0		0
	A_2B	3	0		0
	AB	30	0		0
5 to 6	A_1B	30	0		0
	A_2B	6	0		3
	AB	3	0		0
7 to 8	A_1B	46	1		0
	A_2B	22	0		12
	AB	11	0		0

Table 2
Grouping results from blood stains stored under various conditions

	Type of Stain A_1B	A_2B	A_2B	A_2B	AB
Dry -20 C to 52 C 1 to 10 weeks	✓	✓	✓	✓	✓
Moist -20 C, 4 C, RT 1 to 10 weeks	✓	✓	✓	✓	✓
Moist 37 C, 52 C 1 to 2 weeks	✓	✓	✓	✓	✓
Moist 37 C, 52 C 6 weeks	✓	B	✓	*	✓
Moist 37 C, 52 C 10 weeks	✓	B	B	*	✓

* denotes low levels of A antigen
✓denotes correct grouping.

Of the forty group AB blood stains stored at room temperature for one year, one stain repeatedly typed as a group B. This blood had not been subtyped.

Of the five to six year old stains three out of six A_2B blood stains had lost group A antigenicity and were typed as group B.

Of the seven to eight year old stains, twelve of a total of twenty-two A_2B stains were incorrectly typed as group B.

One of forty-six A_1B stains in the seven to eight year old category had lost B antigenicity and was typed as a group A.

None of the stains that had been typed after storage at room temperature for one to eight years had entirely lost antigenicity.

The results of the stains stored either dry or moist at varying temperatures are shown in table 2.

Three of the stains used in this study were A_2B, one was A_1B and the other was not subtyped as it was received in a lysed condition.All the stains stored dry at temperatures from -20 C to 52 C were typed correctly, with no loss of antigenicity, as shown on the top line of results in table 2. The stains that were stored moist at -20 C, 4 C and at room temperature were also typed correctly with no loss in antigenicity over the ten week period. At the higher temperatures (of 37 C and 52 C), the stains kept moist typed correctly for the first two weeks, but after six weeks there was noticeable loss of A antigenicity in the three A_2B stains. The A_1B and AB (sample not subtyped) were grouped correctly when kept moist at all the temperatures tested.

The three group O blood stains that were similarly stored over a ten week period did not give any cross-reactions. But H antigenicity was lost at elevated temperatures, particularly when kept moist at 52 C when no reactions were obtained even after one day.

In our laboratory saliva stains are grouped by testing dilutions of the stain extract by both absorption inhibition and absorption elution techniques. Thereby a series of agglutination scores are obtained for each of the three, A, B and H antigens.

A simple mathematical method has been devised, such that the amount of each antigen detected is represented by a single number. Direct comparison of the level of the three antigens is then made possible.

Agglutination is scored in our laboratory using a nine point scheme, -, +, 1 onwards to 4 (total agglutination). For the inhibition tests each antigen is tested on three dilutions of the original extract. Table 3 shows conversion figures for agglutination scores obtained for each dilution. The score at each dilution is weighted such that if inhibition is obtained at the third dilution the score will be greater than if inhibition is only obtained from the first dilution.

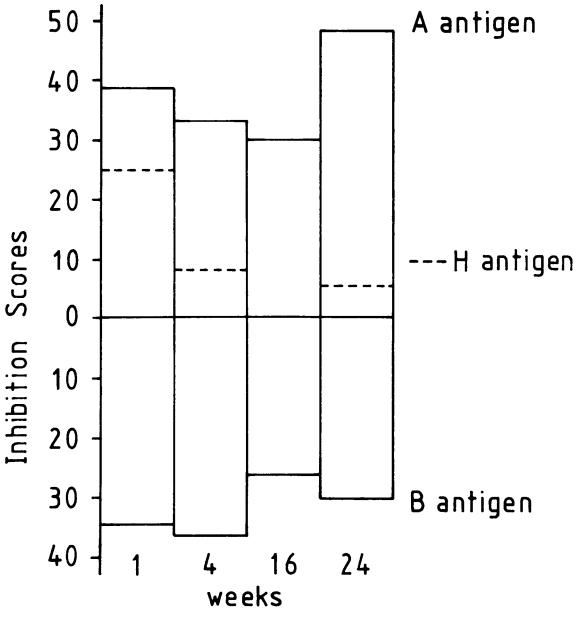
Table 4 shows the conversion chart for the absorption elution method. Here again the scores are weighted such that positive reactions at greater dilutions score more highly.

Consider a result from a group AB saliva stain, such as is given in table 5.

Each agglutination score is converted and then the total for each antigen represented by a single score, which can then be graphically represented.

The five group AB saliva stains used in this preliminary study did not show significant differential levels of A and B antigen. The H antigen, however, was greatly reduced with age. The results from one of these stains which was typical is given in figure 1.

Figure 1. An example of the levels of A, B and H antigen from a group AB secretor saliva stain, represented by converted scores.



The agglutination scores have been converted and the levels of A, B and H antigens detected by the inhibition method are represented over a period of six months.

Conclusions

1) There was a high incidence of loss of A antigen from A_2B blood stains stored at room temperature for over five years.

2) Most group A_1B stains retained their A and B antigenicity for eight years at room temperature. The only exception was one out of forty-six A_1B stains of seven to eight years old which had lost group B antigenicity.

3) In stains stored for ten weeks, loss of A antigenicity was observed only from A_2B stains, and then only when they were stored moist for over six weeks at the more adverse conditions of 37 C and 52 C.

4) The saliva stains used in this study did not show any loss in A or B antigenicity, but only a very small number of stains were typed.

Table 3

Inhibition Score Conversion Chart

Dilution	Score								
Of Extract	-	+	1	1+	2	2+	3	3+	4
1/2	8	7	6	5	4	3	2	1	0
1/5	16	14	12	10	8	6	4	2	0
1/10	24	21	18	15	12	9	6	3	0

Table 4

Elution Score Conversion Chart

Dilution	Score								
Of Extract	-	+	1	1+	2	2+	3	3+	4
1/2	0	1	2	3	4	5	6	7	8
1/5	0	2	4	6	8	10	12	14	16
1/10	0	3	6	9	12	15	18	21	24
1/20	0	4	8	12	16	20	24	28	32
1/40	0	5	10	15	20	25	30	35	40

Table 5

Example of an Inhibition & an Elution result with converted score totals for an AB sec. saliva stain.

	INHIBITION			ELUTION		
	A	B	H	A	B	H
Agglutination	-	-	2+	3	3+	3+
scores	-	-	3	3+	3+	3
	-	+	3+	3+	3+	2+
				3+	3+	2
				3	3+	-
Converted						
scores	48	45	10	99	105	50