ABO grouping studies of human seminal stains on fabrics

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SEMINAL STAIN is a useful physical evidence in a sexual crime. It is therefore important to identify its presence on garments worn by the victim and the accused at the time of the offence and also, if possible, at the place where the offence was committed. These stains would be even more valuable as physical evidence if they could be further characterised so as to establish their origin by grouping the ABO blood group substances in them.

The identification of seminal stains on garments and other articles is well documented.(2,4, 7, 8)

Nickolls has stated in a book (8) that all persons can be divided into two categories, namely those that secrete their group specific ABO agglutinogens in their body exudates and those that do not secrete these substances. He further states that the concentration of agglutinogens in the seminal fluid of secretors is approximately four times as strong as the concentration in their blood and as such, they could be readily grouped using the absorption-inhibition technique which he described earlier in the same volume for blood grouping. However, he makes no mention about seminal stains from non-secretors.

Nickolls and Pereira (9) have stated that they found their modified absorption-elution technique of grouping blood group substances works satisfactorily for saliva and semen.

Outteridge (10) has, in a recent article, mentioned that secretions of some 75 – 80% of individuals contain water soluble blood group substances and these blood group substances could be identified by a simple qualitative absorption test.

Surinder Singh and Aw Yong Heng Khuan (12), too, have used the absorption inhibition technique successfully in grouping seminal stains from secretors. They reported that they were unable to get results in the case of non-secretors by using the same technique.

Friedenreich and Hartman (11) studied the problem of secretors and non-secretors deeply and came to the conclusion that there were two distinct forms of the antigens:

(i) A water soluble form not present in the red cells or serum but present in most of the body fluids and organs of a secretor. The presence of this water soluble antigen is

determined by the secretor gene;

(ii) An alcohol soluble form of the antigen, present in all tissues (except the brain) and in the red cells, but not present in the secretions. The alcohol soluble form is not influenced by the secretor gene. In the light of this knowledge, it can therefore be inferred that seminal plasma from secretors would contain water soluble ABO blood group substances and their sperms would contain both water soluble and alcohol soluble ABO blood group substances, whereas seminal plasma from non-secretors would contain no ABO blood group substances but their sperms would contain only alcohol soluble ABO blood group substances.

Experimental

The experimental work was divided into three parts, namely:-

Part I: The formulation of a suitable routine technique for grouping the ABO blood substances in seminal stains from both secretors and non-secretors.

Part II: To investigate if any modifications were required in grouping the ABO blood group substances in seminal stains on a few variety of fabrics.

Part III: To investigate grouping of the ABO blood group substances in seminal stains from oligospermic and azoospermic individuals.

PartI: Samples of semen for this investigation were donated by members of the staff. In all, 30 samples from 30 individuals (6 group A secretors, 6 group B secretors, 6 group 0 secretors, 2 group AB secretors, 3 group A non-secretors, 3 group B non-secretors, 3 group 0 non-secretors and 1 group AB non-secretor) were examined (all these individuals had more than 200,000,000 sperms in a single ejaculate). The semen were absorbed on cotton fabrics and allowed to dry at room temperature (70°– 80° F).

The secretor or non-secretor status of the individuals were determined from their saliva using the technique described by Race and Sanger (11).

Commercially purchased "Ortho" anti-A and anti-B anti sera were used and they were found to have a titre of 256. The anti-H was obtained from Ulex europaeus seeds according to the method described by Boorman and Dodd (1) and it was found to have a titre of 128. Isotonic saline was used for dilutions of all the above anti sera.

Several variations of the absorption-inhibition (3) and absorption-elution (5) techniques were tried with the following considerations:-

(i) reliability for interpreting results

(ii) adaptability as a routine technique in a busy laboratory.

Part II: Samples of semen for this investigation, too, were donated by members of the staff. The semen were absorbed on nylon, terylene and wool fabrics respectively and allowed to dry at room temperature (70°– 80°F). The successful grouping techniques of Part I were used to determine the ABO blood group substances of seminal stains on the above three fabrics with the view to determine if any further modifications were necessary, dependent on the fabric.

Part III: Samples of semen for this investigation were donated by the Pathology Department of the University Hospital, University of Malaya. In all, 25 samples, made up of 10 azoospermic and 15 oligospermic semen, were examined. The samples of semen were absorbed on cotton fabrics and allowed to dry at room temperature (60°F). The successful grouping techniques of Part I were used to determine the ABO blood group substances of the above seminal stains.

Results

Part I: It was found that seminal stains from secretors could be unequivocally grouped by a simple absorption – inhibition method. However, seminal stains from non-secretors could not be grouped by this same technique. The absorption-inhibition method found reliable and suitable for routine purposes was as follows:-

(i) a piece of stained fabric about 1 sq. cm. was cut into two equal halves and placed in two small dryer's tubes, marked S1 and S2 respectively. Likewise, a piece of similar unstained fabric of 1 sq. cm. was cut into two equal halves and placed in two dryer's tubes, marked B1 and B2 respectively.

(ii) using pasteur pipettes, three drops of an equal mixture diluted anti-A and anti-B (1:16) were added to tubes S1 and B1 resectively and two drops of diluted anti-H (1:8) were added to tubes marked S2 and B2 respectively.

(iii) the absorption was allowed to proceed overnight (approximately 16 hours) at room temperature (60° F)

(iv) as much extract as possible were removed from the above four tubes (by means of suction, using pasteur pipettes with rubber teats) and trans-

Table I

Blood group of donor	Secretor or non-secretor	Agglutination of the serial 2-fold dilutions					
		Slide SA	Slide SB	Slide SO	Slide BA	Slide BB	Slide BO
A	secretor	- - - -	+ + + +	- - - -	+ + + +	+ + + +	+ + + +
B	secretor	+ + + +	- - - -	- - - -	+ + + +	+ + + +	+ + + +
O	secretor	+ + + +	+ + + +	- - - -	+ + + +	+ + + +	+ + + +
AB	secretor	- - - -	- - - -	- - - -	+ + + +	+ + + +	+ + + +
A	non-secretor	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
B	non-secretor	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
O	non-secretor	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
AB	non-secretor	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +

Key:- + represents agglutination
- represents no agglutination

"Slide SA" shows agglutination results of serial 2-fold dilutions to 4 places of the equal mixture of anti-A and anti-B sera afer absorption by the semen stained fabric with group A2 red cells.

"Slide SB" shows agglutination results of serial 2-fold dilutions to 4 places of the equal mixture of anti-A and anti-B sera after absorption by the semen stained fabric with group B red cells.

"Slide SO" shows agglutination results of serial 2-fold dilutions to 4 places of the anti-H serum after absorption by the semen stained fabric with group O red cells.

Slides BA, BB and BO correspond to Slides SA, SB and SO respectively but they show the corresponding results on an unstained fabric.

ferred to fresh dryer's tubes and centrifuged.

(v) the supernatant of the extract from tube S1 was divided into two equal aliquots and each aliquot was serially 2-fold diluted to 4 places on to 4 cavity slides, marked SA and SB respectively. The supernatant of the extract from tube S2 was serially 2-fold diluted on to another 4 cavity slide marked SO.
The extracts from tubes B1 and B2 were correspondingly similarly treated to extracts from tubes S1 and S2 and serially diluted on to 4 cavity slides marked BA, BB and BO respectively.

(vi) using platinum loop, fresh group A2 indicator red cells were added to the dilutions in the 4 cavities of slides SA and BA respectively. Likewise, fresh group B indicator red cells were added to the dilutions on slides SB and BB respectively and fresh group O indicator red cells were added to the dilutions on slides SO and BO respectively.

(vii) all the above slides were placed in a moist chamber and after about half an hour standing, the slides were examined for agglutination under a microscope (x 40 magnification).

Typical agglutination results obtained from secretors and non-secretors of the four different groups are shown in table I.

It was further found that seminal stains from non-secretors could be unequivocally grouped by a simple absorption-elution method similar to that described by Nickolls and Pereira (9). However, similar success was not obtained for grouping of seminal stains from secretors by this same technique.

The absorption-elution method found reliable and suitable for grouping seminal stains from non-secretors was as follows:-

(i) about 2 or 3 threads of approximately 1 cm. long were cut from the stained area of the fabric and placed into each of the 3 cavities of a slide marked S1. Likewise, threads from an unstained area of the same fabric were cut and placed into each of the 3 cavities of a slide marked B1.

(ii) one drop of the 'Ortho' anti-A serum was added to each of the first cavities of slides S1 and B1 respectively and one drop of 'Ortho' anti-B serum was added to each of the second cavities of the above two slides respectively and one drop of anti-H serum was added to each of the third cavities of the above two slides respectively. The threads in the various cavities must be completely immersed in their respective anti-sera.

(iii) absorption was allowed to proceed for at least 4 hours at room temperature (60°F).

(iv) the excess anti sera were removed as completely as possibly by mild suction with a water pump furnished with a pasteur pipette at its end.

(v) ice cold saline was added into each of the cavities containing the threads and the saline removed as in step (iv). This process was repeated 6 – 10 times. The exact number of washings can only be decided by experience and it would vary from one worker to another. It would be prudent to subject the threads on slide B1 to at least one washing less than the threads on slide S1. In this way, agglutination results read subsequently could definitely be interpreted as that not due to underwashing.

(vi) the threads from slide S1 were removed and placed in 3 corresponding cavities on a fresh slide marked S2. Similarly the threads from slide B1 were removed and placed in 3 corresponding cavities of another fresh slide marked B2.

(vii) one drop of 0.2% suspension of fresh group A2 red cells (in saline) was added to each of the first cavities of slides S2 and B2 respectively, and one drop of 0.2% suspension of fresh group B red cells (in saline) was added to each of the second cavities of slides S2 and B2 respectively, and one drop of 0.2% suspension of fresh group O red cells (in saline) was added to each of the third cavities on slides S2 and B2 respectively.

(viii) the slides S2 and B2 were placed in a dry chamber and incubated in an oven at 55°C for 15 minutes.

(ix) after incubation, the slides S2 and B2 were transferred into a moist chamber and rotated on a rota-test.

(x) after about half an hour, the slides were examined for agglutination under a microscope (x 40 magnification).

Typical agglutination results obtained from secretors and non-secretors of the four different groups are shown in table II.

Part II: It was found that the same two techniques described under Results of Part I could be successfully used for grouping seminal stains from secretors and non-secretors respectively on nylon, terylene and wool fabrics. It was, however, found that it was desirable to reduce the number of washings in step (v) of the absorption-elution method for seminal stains from non-secretors on nylon and terylene fabrics. No further modifications were found to be necessary.

Part III: Agglutination results obtained for the abnormal seminal stains using the absorption – inhibition method described under Results Part I are shown in table III.

Discussion

It was found that no single grouping technique could be used to successfully group seminal stains from both secretors and non-secretors. Seminal stains from non-secretors could not be grouped by the absorption-inhibition technique, probably because of the comparative poor sensitivity (10) of the technique. Only the sperms portion of the semen of a non-secretor would contain the blood group substances, and these represent less than 10% of the semen in human beings (6). However, seminal plasma and sperms from secretors contain blood group substances and as such, there are sufficient blood

Table II

Blood group of donor	Secretor or non-secretor	AGGLUTINATION					
		Slide S2			Slide B2		
		1st cavity	2nd cavity	3rd cavity	1st cavity	2nd cavity	3rd cavity
A	secretor	–	–	–	–	–	–
B	secretor	–	–	–	–	–	–
O	secretor	–	–	–	–	–	–
AB	secretor	–	–	–	–	–	–
A	non-secretor	+	–	–	–	–	–
B	non-secretor	–	+	–	–	–	–
O	non-secretor	–	–	+	–	–	–
AB	non-secretor	+	+	–	–	–	–

Key:- + represents agglutination
– represents no agglutination

"Slide S2 – 1st cavity" shows absorption-elution agglutination results by semen stained fibres of anti-A serum with group A2 red cells.

"Slides S2 – 2nd cavity" shows absorption-elution agglutination results by semen stained fibres of anti-B serum with group B red cells.

"Slide S2 – 3rd cavity" shows absorption-elution agglutination results by semen stained fibres of anti-H serum with group O red cells.

The 3 cavities on slide B2 correspond to the 3 cavities on slide S2 but they show the corresponding results on unstained fibres.

group substances in seminal stains from secretors to be detected by the absorption-inhibition method.

Seminal stains from secretors could not be very successfully grouped by the absorption-elution technique, probably because most of the blood group substances in such semen are in a water soluble form and as such are washed away during the washing process employed in the technique. However, this method worked very satisfactorily for seminal stains from non-secretors because (i) it is very sensitive (10) And (Ii) the blood group substances in the semen of a non-secretor is not in a water soluble form.

Our experimental work, using the absorption -inhibition technique of grouping on 20 seminal stains from secretors and 10 seminal stains from non-secretors, has consistently shown that semen from secretors showed absorption-inhibition of anti-H activity whilst semen from non-secretors showed no absorption-inhibition of anti-H activity. Perhaps this phenomenon stain is from a secretor or non-secretor and thence use the appropriate grouping method described earlier in this article.

Grouping results of semen absorbed on three other fabrics showed that the same grouping techniques could be used without any significant modifications.

The grouping studies of seminal stains from azoospermic and oligospermic individuals showed that such stains from secretors could be readily grouped by the absorption-inhibition method but could not be satisfactorily grouped by the absorption-elution method. These findings are consistent with the theory. However, though the two azoospermic seminal stains from non-secretors did not show grouping results for the absorption-inhibition method, nevertheless they showed grouping results for the absorption-elution technique. These results are probably due to cellular debris in the semen as explained by Outteridge (10).

Table III

Stain No.	Azoospermic/ oligospermic	Agglutination of the serial 2-fold dilutions						Remarks
		Slide SA	Slide SB	Slide SO	Slide BA	Slide BB	Slide BO	
1	azoospermic	++++	++++	----	++++	++++	++++	Group O secretor
2	azoospermic	++++	----	----	++++	++++	++++	Group B secretor
3	azoospermic	++++	++++	++++	++++	++++	++++	non-secretor
4	azoospermic	++++	++++	++++	++++	++++	++++	non-secretor
5	azoospermic	++++	----	----	++++	++++	++++	Group B secretor
6	azoospermic	++++	++++	----	++++	++++	++++	Group O secretor
7	azoospermic	++++	++++	----	++++	++++	++++	Group O secretor
8	azoospermic	----	++++	----	++++	++++	++++	Group A secretor
9	azoospermic	----	++++	----	++++	++++	++++	Group A secretor
10	azoospermic	----	----	----	++++	++++	++++	Group AB secretor
11	oligospermic	++++	----	----	++++	++++	++++	Group B secretor
12	oligospermic	----	++++	----	++++	++++	++++	Group A secretor
13	oligospermic	++++	----	----	++++	++++	++++	Group B secretor
14	oligospermic	----	++++	----	++++	++++	++++	Group A secretor
15	oligospermic	++++	++++	----	++++	++++	++++	Group O secretor
16	oligospermic	----	++++	----	++++	++++	++++	Group A secretor
17	oligospermic	++++	++++	----	++++	++++	++++	Group O secretor
18	oligospermic	++++	----	----	++++	++++	++++	Group B secretor
19	oligospermic	----	----	----	++++	++++	++++	Group AB secretor
20	oligospermic	++++	++++	----	++++	++++	++++	Group O secretor
21	oligospermic	++++	++++	----	++++	++++	++++	Group O secretor
22	oligospermic	----	++++	----	++++	++++	++++	Group A secretor
23	oligospermic	++++	++++	----	++++	++++	++++	Group O secretor
24	oligospermic	++++	----	----	++++	++++	++++	Group B secretor
25	oligospermic	++++	++++	----	++++	++++	++++	Group O secretor

Key:- + represents agglutination
– represents no agglutination.

"Slide SB" shows agglutination results of serial 2-fold dilutions to 4 places of the equal mixture of anti-A and anti-B sera after absorption by the semen stained fabric with group B red cells.

"Slide SO" shows agglutination results of serial 2-fold dilutions to 4 places of the anti-H serum after absorption by the semen stained fabric with group O red cells.

Slides BA, BB and BO correspond to slides, SA, SB and SO respectively but they show the corresponding results on an unstained fabric.

Conclusion

The method of choice for grouping seminal stains from secretors is the absorption-inhibition method and for seminal stains from non-secretors is the absorption-elution method.

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Table IV

Stain No.	Azoospermic/ oligospermic	AGGLUTINATION						Remarks
		Slide S2			Slide B2			
		1st cavity	2nd cavity	3rd cavity	1st cavity	2nd cavity	3rd cavity	
1	Azoospermic	–	–	–	–	–	–	no results
2	Azoospermic	–	–	–	–	–	–	no results
3	Azoospermic	+	–	–	–	–	–	Group A
4	Azoospermic	+	–	–	–	–	–	Group A
5	Azoospermic	–	–	–	–	–	–	no results
6	Azoospermic	–	–	–	–	–	–	no results
7	Azoospermic	–	–	–	–	–	–	no results
8	Azoospermic	–	–	–	–	–	–	no results
9	Azoospermic	–	–	–	–	–	–	no results
10	Azoospermic	–	–	–	–	–	–	no results
11	oligospermic	–	+	–	–	–	–	Group B
12	oligospermic	–	–	–	–	–	–	no results
13	oligospermic	–	–	–	–	–	–	no results
14	oligospermic	+	–	+	–	–	–	Group A
15	oligospermic	–	–	–	–	–	–	no results
16	oligospermic	–	–	–	–	–	–	no results
17	oligospermic	–	–	–	–	–	–	no results
18	oligospermic	–	+	–	–	–	–	Group B
19	oligospermic	–	–	–	–	–	–	no results
20	oligospermic	–	–	–	–	–	–	no results
21	oligospermic	–	–	–	–	–	–	no results
22	oligospermic	+	–	–	–	–	–	Group A
23	oligospermic	–	–	–	–	–	–	no results
24	oligospermic	–	–	–	–	–	–	no results
25	oligospermic	–	–	–	–	–	–	no results

Key:- + represents agglutination
– represents no agglutination.

"Slide S2 – 1st cavity" shows absorption-elution agglutination results by semen stained fibres of anti-A serum with group A2 red cells.

"Slide S2 – 2nd cavity" shows absorption-elution agglutination results by semen stained fibres of anti-B serum with group B red cells.

"Slide S2 – 3rd cavity" shows absorption-elution agglutination results by semen stained fibres of anti-H serum with group O red cells.

The 3 cavities on slide B2 correspond to the 3 cavities on slide S2 but they show the corresponding results on unstained fibres.

Agglutination results obtained for the abnormal seminal stains using the absorption-elution method described under Results Part I are shown in table IV.