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A Comparison of Absorption-Inhibition and Absorption-Elution Methods in the Detection of ABO(H) Antigens Found in Vaginal Samples Submitted in Sexual Offense Cases

Absorption-inhibition is a well-established method for the identification of ABO(H) antigens in human body fluids submitted as evidence in sexual offense cases. Absorption-elution has been proposed [*1*] as an alternative method to inhibition for typing this material. Absorption-elution has several potential advantages over absorption-inhibition, including sensitivity and widespread use.

Elution methods may be able to detect trace amounts of ABO(H) antigens present in vaginal samples. This low antigenic activity may result from the time lapse between deposit and collection, the collection methods, or the original manner of seminal deposit. The increased sensitivity of absorption-elution may detect antigens in the semen of nominal nonsecretors that would not be detected by conventional inhibition methods [*2,3*].

Absorption-elution methods for bloodstains have essentially replaced inhibition methods in routine use. Elution methods for seminal antigens would therefore be compatible with existing laboratory procedures.

Background

The ABO(H) typing of vaginal samples from sexual assault victims by absorption-inhibition has been a part of this laboratory's standard analytical program. Although the results are sometimes of little immediate value to the investigators of these cases (for example, only H antigenic activity is found in vaginal samples from a Type-O secretor), the results have frequently been of sufficient value to warrant the continued routine typing of vaginal samples (Table 1). In 117 cases in which the secretor status of the victim was known, foreign antigenic activity was found in 32 (27%).

The laboratory maintains a chronological record of typing results on sexual offense cases, and investigators frequently consult this record for additional information to determine if cases of similar circumstances are otherwise related. Three major series of sex crimes, each involving multiple jurisdictions, have been correlated with the help of this laboratory service in the past two years.

As a part of the standard laboratory practice of confirming results with an independent analysis by a second analyst, we had tried absorption-elution on casework several times with equivocal results. In our testing of these case samples additional antigenic activity

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TABLE 1—*ABO(H) typing by absorption-inhibition in sexual assault cases.*

Cases examined (1976)	187
Cases in which no semen was found	45
Cases processed for antigenic information	142
Cases in which victim's type was not known	25
Cases in which victim's type was known	117
Cases in which no antigenic activity was found	15 (13%)
Cases in which antigenic activity was consistent with victim's standards	70 (60%)
Cases in which antigenic activity was found in addition to victim's	32 (27%)

was often found by absorption-elution that had not been detected by absorption-inhibition. Since the typing was usually done without knowledge of the blood type and secretor status of the perpetrator it was difficult to interpret a difference in results between absorption-inhibition and absorption-elution. The absorption-elution method may have been detecting low antigenic activity in the seminal deposit that had become diluted by the collection method, low antigenic activity in vaginal/seminal secretion of a nonsecretor, or antigenic activity caused by bacteria or other contaminants.

The difficulty in interpreting the results on these few case samples led us to perform the following comparison of absorption-inhibition and absorption-elution typing on evidence specimens collected from sexual offense victims.

Materials

Twenty-five vaginal aspirates, 23 of which contained semen, from evidence submitted in sexual assault cases were used. These saline aspirates of approximately 8 to 10 ml volume were obtained by physicians as part of their routine examination of the alleged rape victim. Most aspirates were examined initially within three days of collection. Each aspirate was divided into four aliquots and stored in cork-stoppered, glass, 10 by 75-mm culture tubes. Two aliquots were immersed in a boiling water bath for 10 min; one set of a boiled and a nonboiled aliquot was stored at room temperature and the other set was stored at 4°C.

Anti-A and anti-B antiserum was supplied by Dade Division, Scientific Products, Miami, Fla. Lectin H was prepared according to the method of Kind [*4*, pp. 354–355], with a titer of approximately 32. Indicator cells (A, B, and O) and bovine serum albumin, 30%, were supplied by Hyland Laboratories, Division of Travenol, Costa Mesa, Calif.

Each aliquot of each aspirate was analyzed by absorption-elution at the time of preparation and at one week, one month, and two to three months, and by absorption-inhibition on the day of preparation and after the last absorption-elution (two to three months).

Methods

Absorption-Inhibition

Anti-A and anti-B antisera and lectin H were previously titered under analysis conditions so the optimal dilutions were known. Each was used in a three-tube dilution for all samples, for example, anti-A antiserum at 1/30, 1/60, and 1/120. Hyland Laboratories A, B, and O indicator cells were washed once and used at a concentration of 0.5 to 1% with bovine serum albumin added.

Sample Preparation—Aspirates were centrifuged to remove cellular debris and the supernatant was used for testing. Known saliva was used for positive and negative controls.

Procedure—One drop of each sample was added to one drop of each antiserum dilution in 6 by 50-mm tubes (nine tubes per sample). The tubes were set up in a wooden block

containing 72 holes (Fig. 1) designed for this purpose. Six unknown samples and positive and negative controls could be analyzed simultaneously.

The sample/antiserum mixtures were incubated at 4°C for a minimum of 4 h. The contents of each tube were then poured into an agglutination plate well, one drop of appropriate indicator cells (A_1, B, or O) was added, and the plate was placed on a rotary shaker. The plates were read at 5 and 15 min. Total inhibition evidenced by no agglutination in the three antiserum dilutions demonstrated the presence of the antigen, while inhibition of one or two of the weaker antiserum dilutions was taken as an indication, but not a demonstration, of the presence of that antigen.

Agglutinations were scored here and in absorption-elution after the protocol of Dunsford and Bowley [*4*, pp. 270-271).

Absorption-Elution

Sample Preparation—A modification of the method of Nickolls and Pereira [5] was used. Two drops of each aspirate aliquot were added to 1-cm threads in a spot plate well and allowed to dry overnight at room temperature in a protected area. All threads were from the same source, Johnson & Johnson cotton gauze.

Procedure—One or two threads were teased in one drop of the appropriate neat antiserum (anti-A, anti-B, and lectin H previously described) in agglutination plate wells; the plates were then incubated in a moist chamber at 4°C for a minimum of 4 h. The threads were rinsed once in a beaker of cold saline and transferred to clean, cold agglutination plates, and three to four more cold saline washes were made with vacuum-assisted aspiration by a finely drawn Pasteur pipette. After the final wash two drops of the appropriate indicator cells (0.5 to 1% concentrations with bovine serum albumin) were added to each well and the preparations were eluted in a 57°C water bath for approximately 20 min. The plates were then placed on a rotary shaker and agglutination was scored at 10 and 30 min. Stirring of the thread in the well was frequently necessary to facilitate agglutination. Positive, negative, and substrate controls were run with each unknown sample.

Pereira et al [*6*] discussed the variability of absorption-elution typing of body fluids. To

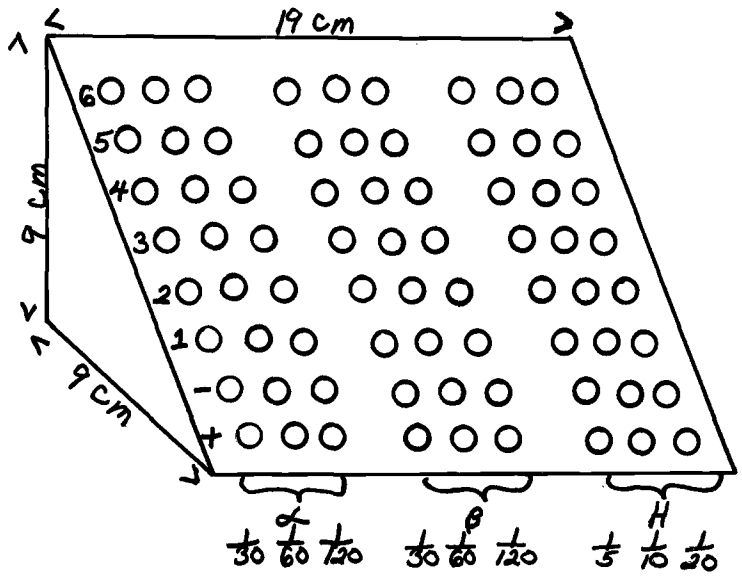

FIG. 1—*Absorption-inhibition block.*

determine if our method was satisfactory for this study, the following modifications of the method had been tried: (1) fixing the dried stain with methanol and (2) teasing the stained threads in saline prior to the addition of antiserum. It was concluded that in our hands our customary absorption-elution method was at least as sensitive as either of these modifications. The postulated "prozone" phenomenon [7] may not be present in our case samples because there is a dilution of antigenic activity in the vaginal aspirate caused by the saline introduced into the vagina during collection.

Results and Discussion

Twenty-five aspirates were analyzed, and the results are summarized in Table 2 and detailed in Table 3. In the first absorption-inhibition analysis, five of the aspirates showed foreign antigenic activity in addition to that activity expected from the known blood type and secretor status of the victim. Thus in 20% of these cases the typing of vaginal aspirates gave positive information potentially useful as evidence.

The results of the absorption-inhibition portion of this study were as expected. The only change in antigenic activity noted with time was a loss of activity in ten aspirates. Since this loss of activity was more pronounced in the nonboiled samples, it may, in part, be a reflection of endogenous or bacterial enzymatic breakdown of the antigenic sites of activity.

On the initial absorption-elution analysis, two aspirates showed an increased number of antigens detected over the comparable absorption-inhibition analysis, which may be the result of increased sensitivity or nonsecretor antigens.

The results of the time study on absorption-elution typing of aspirates were not so readily interpreted. In addition to some loss of activity with time, there was also a random occurrence of additional activity in nine of the aspirates. As Table 3 shows, this additional activity was not always present in consecutive analyses nor did it appear to correlate with method of sample preparation. The parameters involved in absorption-elution typing were kept as constant as possible.

1. Positive and negative controls of known bloodstains were analyzed with expected results with each aspirate analysis.
2. The same antisera lots were used throughout the study.
3. The same substrate source was used to prepare threads.
4. The same analyst performed all work on one aspirate throughout the study, with the exception of occasional vacation conflicts.

The loss of activity in absorption-elution was primarily H activity. Our experience with our method of absorption-elution typing of bloodstains is that the sensitivity for H detection is less than that for A or B, and that sensitivity is more variable. This may be the reason for the greater loss of H detection over A or B.

We have no explanation for the apparent random appearance of A or B antigens in some of the samples. We think that the reproducibility of absorption-elution analysis for ABO(H) antigens in bloodstains may not necessarily be assumed to apply to the analysis of other physiological fluids.

TABLE 2—*Summary of results comparing absorption-inhibition and absorption-elution typing methods.*

Result	Absorption-Inhibition	Absorption-Elution
No antigenic activity on first analysis	4	5
Antigen consistent with victim only on first analysis	16	13
Foreign antigen on first analysis	5	7
Additional foreign antigen in time studies	0	9

TABLE 3—*Results of absorption-elution and absorption-inhibition analyses with time compared to initial absorption-inhibition.*[a]

Sample	Victim's Type	Preparation	Absorption-Inhibition, Initial	Absorption-Elution				Absorption-Inhibition, End of Study
				1 Day	1 Week	1 Month	2-3 Months	
1	A, secretor	1	A,H	A,H	A,H	A,B,H	A,B,H	A,H
		2	A,H	A,H	A,H	A,H	A,H	A,H
		3	A,H	A,B,H	A,B,H	A,H	A,B,H	A,H
		4	A,H	A,B,H	A,H	A,H	A,H	A,H
2	O, secretor	1	A,H	A	A	A,H	neg	neg
		2	A,H	ND	A,B	A,B,H	A	neg
		3	A,H	ND	neg	A,H	A	neg
		4	A,H	ND	A	A	A	neg
3	A, unknown	1	A	neg	neg	A	A	neg
		2	A	neg	neg	A	A	neg
		3	A	neg	neg	ND	A	neg
		4	A	neg	neg	neg	A	neg
4	O, secretor	1	A,H	A,H	A,H	A,H	A,H	A,H
		2	A,H	ND	A,H	A,H	H	A,H
		3	A,H	ND	A,H	A,H	A,H	A,H
		4	A,H	ND	A,H	A,H	A,H	A,H
5	O, secretor	1	H	H	neg	A	neg	H
		2	H	ND	neg	neg	neg	H
		3	H	ND	neg	H	neg	H
		4	H	ND	neg	H	neg	H
6	O, unknown	1	H	ND	H	neg	B	H
		2	H	ND	H	neg	B,H	H
		3	H	ND	H	H	A,B,H	H
		4	H	ND	H	H	B	H
7	O, secretor	1	H	H	H	H	neg	H
		2	H	H	H	neg	neg	H
		3	H	H	H	H	neg	neg
		4	H	H	H	H	neg	H
8	A, nonsecretor	1	neg	neg	A	neg	neg	neg
		2	neg	neg	A,H	neg	neg	neg
		3	neg	neg	A	neg	A	neg
		4	neg	neg	A,H	neg	A	neg
9	A, secretor	1	A,H	A,H	A,H	A,H	A,B	A,H
		2	A,H	A,H	A,H	A	A	A
		3	A,H	A,H	A,H	A,H	A,B,H	A,H

TABLE 3—*Continued.*

Sample	Victim's Type	Preparation	Absorption-Inhibition, Initial	Absorption-Elution				Absorption-Inhibition, End of Study
				1 Day	1 Week	1 Month	2–3 Months	
		4	A,H	A,H	A,H	A,H	A,B	A,H
10	O, secretor	1	H	neg	H	neg	B,H	H
		2	H	H	H	neg	H	H
		3	H	H	H	neg	H	H
		4	H	H	H	neg	H	H
11	A, secretor	1	A,H	A,H	A,H	A	A,H	A
		2	A,H	A,H	A,H	A	A	A
		3	A,H	A,H	A,H	A	A	A
		4	A,H	A,H	A,H	A,H	A,H	A
12	O, unknown	1	neg	A	A	A	A,B	neg
		2	neg	neg	neg	neg	B	neg
		3	neg	A	neg	neg	A,B	neg
		4	neg	neg	neg	neg	B	neg
13	O, unknown	1	neg	neg	neg	neg	neg	neg
		2	neg	neg	neg	neg	neg	neg
		3	neg	neg	neg	neg	neg	neg
		4	neg	neg	neg	neg	neg	neg
14	A, unknown	1	A,H	A	A	A	A	A,H
		2	A,H	A	A	A	A	A,H
		3	A,H	A	A	A	A	A,H
		4	A,H	A	A	A	A	A,H
15	O, secretor	1	A,H	A,H	A	A	neg	A,H
		2	A,H	A,H	A	A,H	A,B	A,H
		3	A,H	A	A	A	A	A,H
		4	A,H	A,H	A	A	A,B,H	A,H
16	A, secretor	1	A,H	A,H	A	A	A	A,H
		2	A,H	A,H	A	A	A	A,H
		3	A,H	A,H	A	A	A,H	A,H
		4	A,H	A,H	A	A	A	A,H
17	O, unknown	1	A,H	A,H	A,H	A,H	H	A,H
		2	A,H	A	neg	A,H	H	A,H
		3	A,H	A,H	neg	A,H	A,H	A,H
		4	A,H	A,H	A	H	A,H	A,H
18	O, nonsecretor	1	H	neg	neg	neg	neg	H

		2	H	neg	neg	neg	neg	H
		3	H	neg	neg	neg	neg	H
		4	H	neg	neg	neg	neg	H
19	O, secretor	1	H	H	H	A,H	A	neg
		2	H	H	H	H	H	neg
		3	H	H	neg	H	H	neg
		4	H	H	H	H	H	neg
20	A, secretor	1	A,H	A,H	A,H	A,H	A,H	A,H
		2	A,H	A,H	A,H	A,H	A,H	H
		3	A,H	A,H	A,H	A,H	A,H	A,H
		4	A,H	A,H	A,H	A,H	A,H	A,H
21	A, secretor	1	H	H	H	H	H	H
		2	H	H	H	H	H	H
		3	H	neg	neg	neg	neg	H
		4	H	H	H	H	H	H
22	A, secretor	1	A,H	A	A	A,H	ND	A,H
		2	A,H	A	A	neg	ND	neg
		3	A,H	A	A	A,H	ND	A,H
		4	A,H	A	A	A	ND	neg
23	A, secretor	1	A,H	A,H	A,H	A,H	ND	A,H
		2	A,H	A,H	A,H	A	ND	neg
		3	A,H	A,H	A,H	A,H	ND	A,H
		4	A,H	A	A	A	ND	H
24	O, secretor	1	neg	neg	neg	neg	ND	neg
		2	neg	neg	neg	neg	ND	neg
		3	neg	neg	neg	neg	ND	neg
		4	neg	neg	neg	neg	ND	neg
25	O, secretor	1	A,H	neg	A	neg	ND	A,H
		2	A,H	A,H	A	neg	ND	neg
		3	A,H	A	A	neg	ND	A
		4	A,H	A,H	A,B	neg	ND	neg

[a] Key

1 = room temperature, boiled
2 = room temperature, nonboiled
3 = refrigerated, boiled
4 = refrigerated, nonboiled
ND = analysis not done
neg = no antigenic activity found

Notes

1. The 25 aspirates used for this study included none from a B secretor.
2. Positive and negative control bloodstains were run simultaneously with all experimental samples and gave the expected results.
3. Foreign antigenic activity noted in a given sample by absorption-elution analysis was not always consistent for each subsequent time period. These inconsistencies may be due in part to variability in sensitivity of absorption-elution typing; however, the authors think that there may be other factors involved.

Conclusions

1. Absorption-elution experiments on threads prepared from vaginal aspirates were not reproducible with time or methods of sample preparation.

2. Results of absorption-elution experiments did not correspond to the absorption-inhibition results on the same samples.

3. Stained threads prepared from vaginal aspirates in the described manner may not be the sample of choice for absorption-elution typing of biological fluids.

Based on these conclusions we feel that, without further study, absorption-elution is not a compatible confirmatory technique for absorption-inhibition in the ABO(H) typing of vaginal material collected in sexual offense cases.

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