Typing of ABH Antigens in Body Fluids by a Two Dimensional Absorption-Inhibition Procedure

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INTRODUCTION

Absorption-inhibition procedures have been used for many years for determining the ABH antigens of dried blood and body fluid samples (Gaensslen, 1983). Although the absorption elution technique originally devised by Siracusa (1923) and refined by Kind (1960) is now used almost exclusively for ABH antigen typing of bloodstains, inhibition remains the method choice in many laboratories for determination of the soluble ABH substances in secretor body fluids and body fluid stains.

There are three categories of inhibition techniques in common use: the one-step or all-or-none; inhibition titration (Holzer, 1937); and titration inhibition (Hirszfeld and Amzel, 1932; Kind, 1955). The sensitivity of the one-step procedure is inversely related to the titer of the antisera used for testing. The titration methods have been adopted in a number of laboratories to try to give a more conclusive indication of the antigen content of questioned samples given the range of variation of ABH antigen concentrations in different secretor body fluids, and the range of variation of concentrations of these antigens in stain extracts. The inhibition titration (IT) and titration inhibition (TI) procedures differ in sensitivity. One way of demonstrating these differences is examining the types of agglutination results one would expect to obtain under test conditions where sufficient antigen is present in the sample to remove a defined quantity (1/2, 1/4, 1/8, etc.) of available antibody. When this analysis is done, it can be shown that TI is more sensitive than IT. In a five tube test with an initial antiserum titer of 1:32, for example, a quantity of antigen sufficient to bind half the available antibody would result in a one tube reduction using IT, but would result in a four tube reduction using TI, as compared with the saline control row. A number of workers prefer to observe a three or more tube reduction with a titration technique before considering the result conclusive. The smallest quantity of antigen in a sample that will still give a three tube reduction by TI is an amount sufficient to bind 1/4 of the available antibody. This same quantity of antigen would yield no inhibition in an IT test, and it would give ambiguous results at best in a one step test.

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The two dimensional procedure described here takes maximum advantage of the best features of both TI and IT, and can be shown to be more sensitive than either TI or IT alone.

METHODS

The two dimensional absorption inhibition procedure consists of first constructing a TI test protocol, then treating each tube in the row as though it were the first tube in an IT test. The complete test protocol is illustrated in Fig. 1 with an initial antiserum titer of 1:128. In practice, a lower initial titer (usually 1:32) is used. Further, the test can be carried out using three or four selected antiserum dilutions without sacrificing sensitivity, which is the technique's principal advantage (Fig. 2). This approach simplifies the protocol and reduces the time required to carry out the test in routine casework. An additional simplification involves setting up the first row with two volumes of sample and antisera, then removing two volumes to another row for possible titration in the second dimension (Fig. 3). Test cells are then added to the original tubes and the results read (essentially a TI test) before proceeding. If the results are conclusive, it is not necessary to carry out the remainder of the procedure.

RESULTS and DISCUSSION

The two-dimensional (2D) absorption inhibition procedures has been used to test a number of known control secretor body fluids and stains of potential forensic interest including 36 salivas, 9 semens, 60 urines, 43 urine stains and 39 perspiration stains representing all four ABO blood groups. The tests were run in parallel with the other three established procedures for carrying out inhibition tests. Donors of the known control samples were classified as secretors on the basis of TI tests on their saliva. Table 1 shows the results of the comparative study. A result is classified as "incorrect" if the test failed to detect one or more antigens known to be present and where this failure would have resulted in the incorrect classification of secretor status or in an incorrect classification of the ABO group of origin. A titration technique result is classified as "inconclusive" if it failed to give convincing reduction of the antibody content relative to the saline control (less than three tubes).

Many incorrect results with all samples were recorded using the one tube technique. By its nature, the procedure cannot be expected to yield reliable detection of soluble antigens in secretor body fluids which are known to have a wide range of concentrations of the blood group substances. With saliva and semen, IT gave no incorrect results but seven salivas and two semens yielded a inconclusive results. TI gave uniformly correct results with these samples, and no inconclusives, illustrating the greater sensitivity of TI as compared with IT. And, for the saliva and semen samples tested, TI was as effective as 2D.

Table 1. Results Obtained on Known Control Samples with the Four Inhibition Techniques

Type of Sample	ABO Group	N	Incorrect (X) or Inconclusive (INC) Results Obtained Using							
			One Tube		I-T		T-I		2-D	
			X	INC	X	INC	X	INC	X	INC
Secretor Salivas	A	13	1	na	0	2	0	0	0	0
	B	7	1	na	0	1	0	0	0	0
	O	9	2	na	0	1	0	0	0	0
	AB	7	3	na	1	3	0	0	0	0
Secretor Semens	A	2	1	na	0	1	0	0	0	0
	B	2	0	na	0	0	0	0	0	0
	O	3	0	na	0	0	0	0	0	0
	AB	2	1	na	0	1	0	0	0	0
Secretor Urines	A	19	6	na	5	7	0	1	0	0
	B	15	2	na	0	5	0	1	0	0
	O	15	7	na	1	5	0	1	0	0
	AB	11	4	na	1	2	0	1	0	0
Secretor Urine Stains	A	11	5	na	2	2	0	0	0	0
	B	16	4	na	0	3	0	1	0	0
	O	10	4	na	2	2	0	0	0	0
	AB	6	5	na	4	1	0	1	0	0
Secretor Sweat Stains	A	12	8	na	3	4	0	0	0	0
	B	10	5	na	0	3	0	1	0	0
	O	10	6	na	2	2	0	0	0	0
	AB	7	3	na	2	0	0	0	0	0

na = not applicable

The value of the increased sensitivity of the 2D procedure as compared even with TI, however, is seen in the results obtained for urines and perspiration. These fluids, as is well known, typically have significantly lower concentrations of soluble ABH antigens than the corresponding saliva or semen of secretor individuals. Here, IT gave a number of incorrect results. And although no incorrect results were obtained with TI, a number of samples yielded inconclusive results. The 2D procedure by contrast resolved all of these inconclusives.

The recent increase in drug screening tests on individuals using urine has made the reliable typing of urines as an aid to identification more important. Urine samples thought to have been mislabeled or incorrectly tested for drugs are now often submitted with a request to compare the grouping results with the ABO group and secretor status of the alleged donor of the urine.

In casework, however, even stains of saliva and semen are sometimes difficult to group correctly by conventional techniques, either because their soluble antigen content is intrinsically low or because the quantity of sample available is low, or both. Table 2 shows that in a number of these cases, the 2D technique has enabled us to obtain conclusive results which would have been unobtainable without it.

Table 2. Results Obtained in Problem Case Samples with the Four Inhibition Techniques

Type of Sample	ABO Group and Secretor Status	Results Obtained With One Tube	I-T	T-I	2-D
Saliva	A secr	H	(A),(H)	A,H	A,H
	AB secr	B	(B),(H)	(B),(H)	A,B,H
	AB secr	A	(A),(B)	A,B,H	A,B,H
Semen	AB secr	A,H	A,(B),(H)	A,B,H	A,B,H
	A secr	H	(A),(H)	(A),(H)	A,H

( ) = weak, inconclusive results for the antigen

The 2-D procedure thus provides an additional measure of sensitivity that is especially valuable in resolving the ABH antigen content of samples having ABH antigen concentrations too low to be reliably detected by existing procedures. We have used the technique in conjunction with absorption-elution as a reliable method for grouping bone tissue (see Lee and Gaensslen, these Proceedings). If the procedure suggested in Fig. 3 is followed, the 2-D technique need only be carried to completion when the IT test represented by the first row does not provided conclusive results. This approach conserves valuable examiner time and effort, and thus enables a laboratory to use this technique routinely.

REFERENCES

Gaensslen RE (1983), Sourcebook in Forensic Serology, Immunology and Biochemistry. U.S. Government Printing Office, Washington, D.C.

Hirszfeld L, Amzel R (1932) Beitrag zur gerichtlich-medizinischen Verwertung der Blutgruppen. Dtsch Z Gesamte Gerichtl Med 19: 133-148

Holzer FJ (1937) Untersuchungen uber die gerichtlich-medizinische Verwertbarkheit der Ausscheidung Blutgruppen-substanzen. Dtsch Z Gesamte Gerichtl Med 28: 234-248

Kind SS (1955) A modified absorption technique of determining the ABO group of bloodstains. Vox Sang [O.S.] 5: 15-19

Kind SS (1960) Absorption elution grouping of dried blood smears and Absorption elution grouping of dried bloodstains on fabrics. Nature 185: 397-398 and 187: 789-790

Siracusa V (1923) La sostanza isoagglutinabile del sangue. Arch Antropol Crim Psichiatr Med Leg 43: 362-364

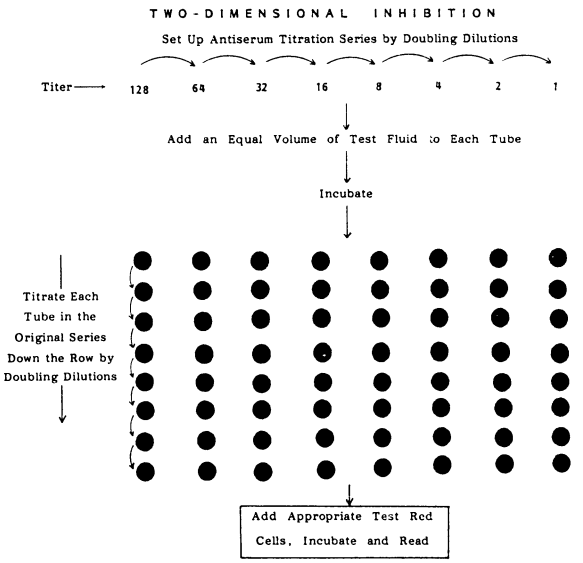

Fig. 1. Overall Two Dimensional Procedure

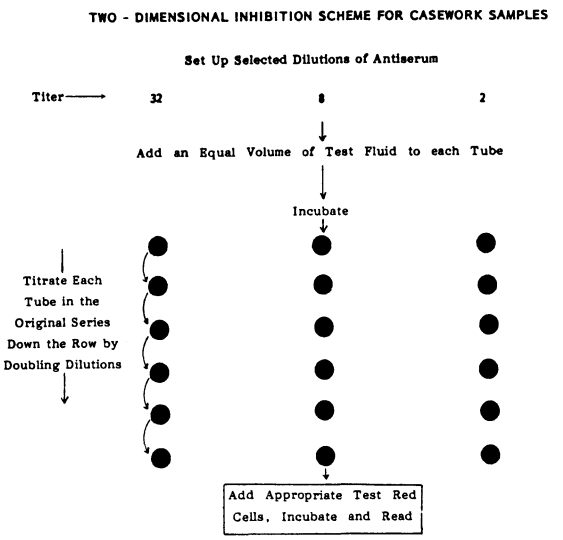

Fig. 2. Two Dimensional Procedure for Casework Using Selected Dilutions of Antisera

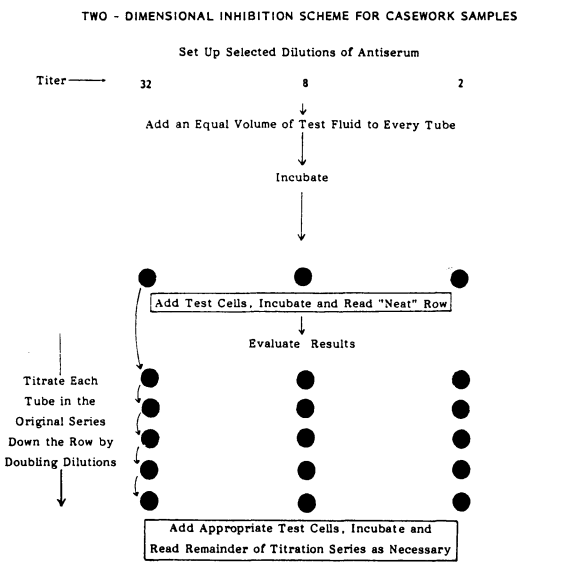

Fig. 3. Two Dimensional Procedure Scheme for Casework in which Results of the TI Dimension are Read Before Proceeding to the IT Dimension