AN ELISA METHOD FOR DETECTING LEWIS ANTIGENS IN BODY FLUIDS

F. Samuel Baechtel and Frances C. Henning
Forensic Science Research Group, FBI Academy, Quantico, Virginia, U.S.A.

Detection of Lewis^{a} (Le^{a}) or Lewis^{b} (Le^{b}) antigens in body fluids enables the prediction or corroboration of the ability of the fluid depositor to secrete soluble ABH substances (1-4). The hemagglutination-inhibition technique, usually employed for the detection of the Lewis antigens, is not well suited for processing large numbers of samples on a routine basis. The genesis of monoclonal antibodies, specific for Le^{a} and Le^{b}, has promoted the development of enzyme-linked immunosorbent assays (ELISA) for the detection of these antigens (5-6). The ELISA described in this paper incorporates these monoclonal reagents into a technique that can be used to efficiently analyze a large number of samples for the presence of Le^{a} and Le^{b} in body fluids.

METHODS AND MATERIALS

The ELISA was configured such that the presence of Le^{a} or Le^{b} in a test specimen could be detected by its ability to inhibit the binding of the appropriate monoclonal antibody to immobilized homologous antigen. The assay was carried out in 96-well, polystyrene microplates (Dynatech Laboratories, Alexandria, Virginia). Lewis blood group substance standard (LBGS), containing both Le^{a} and Le^{b} activity (Ortho Diagnostics, Raritan, New Jersey), was diluted with 0.05 M carbonate/bicarbonate buffer, pH 9.6. The LBGS was diluted 1:3200 and dispensed in 200 uL portions to wells in the upper half of each plate, which were devoted to tests for Le^{a} antigen. Wells in the lower half of the plate received 200 uL LBGS diluted 1:1600. These were used to test for the presence of Le^{b}. After addition of the LBGS, the plate was maintained at 4^{o} C overnight to permit binding of the antigens to the plate.

After antigen immobilization, the plate was washed three times with buffered saline that contained 1% liquid gelatin (GHBS) (Hypure, New Brunswick, New Jersey). Unoccupied binding sites in the plate were blocked by filling all wells with 3% GHBS and incubating for one hour at 37^{o} C. Plates can be sensitized with LGBS, blocked, and stored up to four days in advance of their use.

Body fluid specimens were diluted with 1% GHBS and tested for Le^{a} and Le^{b} contemporaneously on the same plate. One-hundred uL portions of the specimens were added, in duplicate, to wells devoted to tests for Le^{a}; and duplicate 100 uL aliquots added to Le^{b} test wells. Immediately following addition of test specimens, 100 uL monoclonal anti-Le^{a} (1:500 dilution with 1% GHBS) were added to the Le^{a} test wells; and 100 uL monoclonal anti-Le^{b} (1:1000 dilution with 1% GHBS) placed in each of the Le^{b} test wells. Both monoclonal antibodies were obtained from the Genetic Testing Institute, Atlanta, Georgia. Four wells in the Le^{a} test area and four in the Le^{b} area received 100 uL 1% GHBS instead of test specimens. These wells served as no antigen controls in which maximum binding of monoclonal antibody could be assessed. After addition of reagents,

Advances in Forensic Haemogenetics 1
Edited by B. Brinkmann and K. Henningsen

the plate was placed at 37° C for 30 minutes.

After three washes with 1% GHBS, each well received 200 ul of alkaline phosphatase conjugated goat antimouse IgM (GAMIGMAP) (Sigma Chemical Company, St. Louis, Missouri) diluted 1:1000 with 1% GHBS. The plate was incubated one hour at 37° C followed by three washes with 1% GHBS.

The residual alkaline phosphatase activity in the wells was assayed by adding 200 uL p-nitrophenyl phosphate (6.0 mg/ml 0.1 M glycine buffer, pH 10.4, containing 0.001 M MgCl and 0.001 M ZnCl) and incubating at 37° C for 30 minutes. The absorbance in each well at 405 nm was determined in an automated microplate reader.

The mean and standard deviation of the mean were calculated for the absorbancies in the quadruplicate wells which received anti-Le^a or anti-Le^b but no test specimen. Three standard deviations were subtracted from the mean absorbance for each antibody to yield a corrected no antigen control value for each antigen. The reduction of binding of either monoclonal anti-Le^a or anti-Le^b, due to inhibition by antigen, was considered significant if the mean test well absorbance was equal to or less than the appropriate no antigen control value. The titer of Le^a or Le^b in a body fluid specimen was defined as the reciprocal of the greatest dilution that was capable of significant inhibition.

Saliva was obtained from personnel at the FBI Academy. Immediately after collection, each saliva sample was placed in boiling water for five minutes and stored at -70° C until examined for Le^a and Le^b presence. Semen specimens were obtained by regional fertility clinics from normal individuals with no history of genitourinary pathology. Semen specimens were stored at -70° C until tested. Vaginal fluid specimens were obtained on tampons inserted for six hours by donors who refrained from sexual activity for 72 hours prior to sample collection. The tampons were air-dried. Crusted areas were cut and extracted in buffered saline for tests.

RESULTS

For this study, it was desired that an absorbance at 405 nm of about 1.0 be obtained in the no antigen control wells for both Le^a and Le^b after 30 minutes of incubation with substrate. The concentrations of LBGS, the monoclonals to Le^a and Le^b, and the antimouse immunoglobulin conjugate necessary to achieve this final absorbancy were determined by titration. LBGS, diluted 1:3200 was optimal for detection of Le^a antigen; whereas a dilution of 1:1600 was necessary for Le^b detection. The optimal dilution of monoclonal anti-Le^a was shown to be 1:500, and the optimal dilution for the monoclonal anti-Le^b was found to be 1:1000. The GAMIGMAP was used at a dilution of 1:1000.

Figure 1 illustrates the typical inhibition patterns seen when serial dilutions of semen specimens from secretor and nonsecretor individuals were tested for their ability to inhibit the binding of the monoclonal antibodies. This figure points out also the method by which the Le^a and Le^b titers were derived for a given body fluid specimen. Note that nonsecretor semen was capable of significantly inhibiting the binding of monoclonal anti-Le^a up to a 1:80 dilution of the sample. The Le^a titer of this specimen

would be 80 (reciprocal titer). No Le^b activity was demonstrable in this specimen at the minimum diluton tested of 1:20. Semen from a secretor individual significantly inhibited the binding of monoclonal anti-Le^b up to a specimen dilution of 1:1280. The Le^a level in this specimen was low, yielding a reciprocal titer of only 20.

Using this approach, the levels of Le^a and Le^b were determined for saliva specimens from 44 individuals, 20 secretors and 24 nonsecretors. The results are shown in Table 1. The Le^b levels in saliva from secretors varied over more than a 100-fold range, with the mean titer being 4240. The mean level of Le^a in this group was 478, with the actual titers varying between 100 and 3200. Nonsecretor saliva specimens demonstrated the same range of Le^a titers as was seen for Le^b in secretors, with almost the same mean titer. For 7 of the 24 nonsecretor specimens, an apparent Le^b activity was present, although of low titer. Repetitive analyses of these specimens failed to consistently demonstrate Le^b presence. It should be pointed out that the minimum dilution of saliva tested was 1:100. Thus, the presence of either Le^a or Le^b at titers less than 100 was not established.

Table 2 displays the levels of Le^a and Le^b in 68 semen specimens. The magnitude of seminal fluid Lewis titers, for secretors and nonsecretors, was considerably less than that seen for saliva. The mean titer of Le^b in secretor semen was 15-fold less than that seen in secretor saliva. The mean titer of Le^a in nonsecretor semen was almost 30-fold less than nonsecretor saliva. Five of 26 nonsecretors exhibited very low titers of an apparent Le activity. As seen with saliva, the appearance of low titer Le^b activity in nonsecretor semen was unpredictable upon replication. Since the minimum dilution of semen examined was 1:20, the occurrence of Lewis titers less than 20 cannot be ruled out.

This technique was used to test for the presence of the Lewis antigens in vaginal fluid stains from eight donors. The Lewis phenotype of each donor was established by direct test upon the donor's red blood cells. Five of the donors were Le^{a-b+} and the Le^b antigen was demonstrable in stains from each. Two individuals were Le^{a+b-}, but stains from only one donor exhibited Le^a. Neither Le^a or Le^b was demonstrable in one Le^{a+b-} individual. The remaining donor was Le^{a-b-}.

DISCUSSION

The ELISA procedure described in this paper is capable of detecting the Le^a and Le^b antigens in body fluid samples in a relatively short period of time. The test can be completed in fewer than four hours and enables a large number of specimens to be analyzed concurrently. The major portion of the assay time is consumed by the incubation phases.

A comparison of the Le^a and Le^b titer ranges determined by this ELISA, with similar studies reported by others (7-8), indicates that the sensitivity of the current technique equals, but does not exceed, that of hemagglutination-inhibition.

Given the level of sensitivity of this ELISA method, it is important to consider its potential utility for the routine examination of body fluid stains associated with forensic evidence. Considering the magnitude of the Lewis antigen titers in saliva, one would not anticipate difficulty in demonstrating Le^b from secretors or Le^a from nonsecretors, assuming the specimen had sustained an overall dilution of no more than 50- to 100-fold. On the other hand, the much lower Lewis titers observed for semen suggest that these antigens will be detectable only in stain extracts which contain high concentrations of semen.

Despite the modest sensitivity exhibited by this ELISA method it does possess two salient features: (1) it enables the concurrent and expeditious analysis of a larger number of specimens than the conventional hemagglutination-inhibition method; and (2) it utilizes monoclonal antibodies to the Lewis antigens which are of defined specificity and affinity, insuring that this aspect of the assay is invariant. The latter features are unattainable with polyclonal antisera to these antigens.

REFERENCES

1. Pereira, M., and Martin, P.D., Problems involved in the grouping of saliva, semen and other body fluids. J. Forens. Sci. Soc., 16:151, 1976.
2. Pereira, M., and Martin, P.D., Determination of Lewis secretions in body fluids and dried stains of body fluids. J. Forens. Sci. Soc., 17:135, 1977.
3. Piner, S. C., and Sanger, M. S., Lewis grouping of human secretion stains. Forens. Sci. Int., 15:87, 1980.
4. Davie, M. J., Lewis grouping on microtiter plates. J. Forens. Sci. Soc., 20:267, 1980.
5. Messeter, L., Brodin, T., Chester, M. A., Karlsson, K. A., Zopf, D., and Lundblad, A., Immunochemical characterization of a monoclonal anti-Le blood grouping reageant. Vox Sang., 46:66, 1984.
6. Ernst, C., Atkinson, B., Wysocka, M., Blaszczyk, M., Herlyn, M., Sears, H., Steplewski, Z., and Koprowski, H., Monoclonal antibody localization of Lewis antigens in fixed tissues. Lab. Invest., 50:394, 1984.
7. Baer, B. R. and Kearney, J. J., A micro-capillary adsorption-inhibition method for the determination of Lewis phenotypes from semen and saliva stains. Abstract, presented at the 35th Annual Meeting of the American Academy of Forensic Sciences, February 1983, Cincinnati, Ohio.
8. Gibbons, M. M., Distributions of ABH and Lewis blood group substances in vaginal secretions. M. S. Thesis, University of California, Berkeley, 1984.

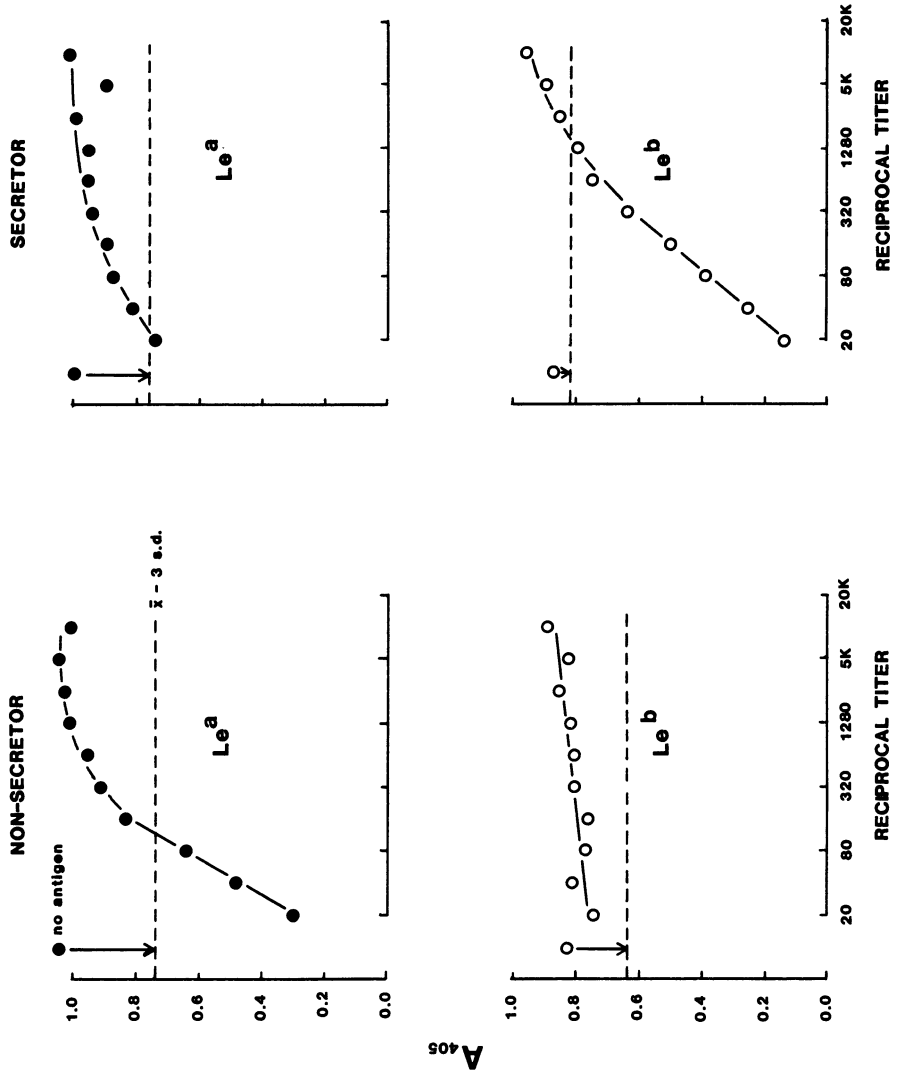

FIGURE 1. INHIBITION OF BINDING OF MONOCLONAL ANTI-LEWIS ^{A} AND ANTI-LEWIS ^{B} BY SECRETOR AND NONSECRETOR SEMEN.

TABLE 1

LEVELS OF LE^A AND LE^B ANTIGENS IN SALIVA [1]

I. SECRETOR

	RECIPROCAL TITER	
	LE^A	LE^B
RANGE:	100-3200	400-51200
MEAN[2]:	478[3]	4240

II. NON-SECRETOR

	RECIPROCAL TITER	
	LE^A	LE^B
RANGE:	400-51200	100-400
MEAN:	4025	200[4]

[1] MINIMUM SALIVA DILUTION TESTED WAS 1/100
[2] GEOMETRIC MEAN
[3] 17/20 SPECIMENS EXHIBITED LE^A ACTIVITY
[4] 7/24 SPECIMENS EXHIBITED LE^B ACTIVITY

TABLE 2

LEVELS OF LE^A AND LE^B ANTIGENS IN SEMEN[1]

I. SECRETOR

	RECIPROCAL TITER	
	LE^A	LE^B
RANGE:	40-160[3]	20-5120
MEAN[2]:	63	288

II. NON-SECRETOR

	RECIPROCAL TITER	
	LE^A	LE^B
RANGE:	20-10240	20-40[4]
MEAN:	150	23

[1] MINIMUM SEMEN DILUTION TESTED WAS 1/20
[2] GEOMETRIC MEAN
[3] 6/24 SPECIMENS EXHIBITED LE^A ACTIVITY
[4] 5/26 SPECIMENS EXHIBITED LE^B ACTIVITY