CASE REPORT

Sara Bolton,[1] *B.Sc. and James Thorpe,*[1] *Ph.D.*

An Examination of a Contaminated Seminal Stain Using Absorption-Elution and Enzyme-Linked Immunosorbent Assay (ELISA)

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ABSTRACT: A semen stain, apparently contaminated with a detergent cleanser, was received for examination. The contamination interfered with the normal biochemical reactions of such stains. Treatment of the sample enabled ABO groups to be determined.

KEYWORDS: forensic science, semen, genetic typing, immunoassay, ELISA

A bed sheet was submitted for examination for the presence of seminal material. This is a common request, but on this occasion the sheet was accompanied by a bottle of "Tabort" cleaner which may have been used in an attempt to remove any staining present.

"Tabort" is a viscous liquid used by applying to the stained area, allowing the liquid to soak into the stain, and then washing with water. The cleanser appears to be a detergent.

Initial Examination

The bed sheet was in good condition and clean apart from two translucent areas close together each approximately 3 cm in diameter.

The sheet was tested for peroxidase activity, using Leuco-malachite green, and for acid phosphatase activity [*1*]. The whole sheet gave negative results. It was noted that the translucent areas were slightly sticky.

A water extract from one translucent area was mounted on a microscope slide, stained with haematoxylin and eosin, and examined. The extract was found to contain a few intact spermatozoa and numerous heads.

The techniques available for grouping were typing of phosphoglucomutase using isoelectric focusing and ABO grouping by absorption-elution (A/E), absorption-inhibition (A/I), or enzyme-linked immunosorbent assay (ELISA). Normally, two of these techniques for

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[1]Research assistant and lecturer in forensic science, respectively, Forensic Science Unit, Strathclyde University, Glasgow, United Kingdom.

ABO grouping would be used upon nonblood body fluid stains. In the absorption-inhibition test, the detergent caused hemolysis of the indicator cells, limiting the choice to absorption-elution and ELISA. Both these techniques require immobilization of the sample. While the detergent may interfere with the binding of the sample, it should be removed before the detection stages by the washing steps used in both these procedures. Any detergent would not therefore cause hemolysis of cells in the A/E procedure.

Materials and Methods

Duplicate stains of semen and saliva were prepared on clean cotton cloth from samples donated by volunteers. One stain of each pair was treated with "Tabort." Samples were extracted in distilled water for isoelectric focusing and the procedure was essentially that of Barke et al. [*2*] but using 0.15-mm-thick gels [*3*].

For ELISA, samples were extracted by mixing 1-cm squares of the stained (or control) fabric in 0.8 mL of pH 12 buffer for 8 h at room temperature. The fabric was removed and debris separated by centrifugation. It has been found that the addition of the pH 12 buffer ensures that all the samples to be assayed have the same pH.[2]

ELISA grouping was carried out by the technique of Bolton and Thorpe [*4*] extended to include anti-H. The anti-A and anti-B were Seraclone reagents from Biotest-Folex (Birmingham, England), the anti-H from Dako Ltd. (High Wycombe, Bucks, England). All these antibodies are immunoglobulin M (IgM) class and were produced in mice. They were detected with goat anti-mouse IgM, conjugated to horse radish peroxidase, from Sigma Ltd. (Poole, Dorset, England).

The absorption-elution procedure was based upon that described by Kind and Cleevely [*5*] modified by using polycarbonate sheet as a support instead of microscope slides. The anti-A and anti-B were polyclonal antisera from Immuno Diagnostics (Seven Oaks, Kent, England). The H lectin was prepared from seeds of *Ulex europaeus* by saline extraction. Absorption was at 4°C overnight followed by three washes, each of 2-min duration, in chilled isotonic saline, blotting dry, and then elution into a drop of saline at 60°C for 30 min.

When it was found necessary to try to remove the cleanser, the method used was that of Horigome and Sugano [*6*]. Amberlite XAD-2 polystyrene beads (BDH Chemicals Ltd., Poole, England) were used in 4-by-10-mm columns. The beads were suspended in pH 12 coating buffer and packed into the columns. These were then centrifuged at 2000 rpm for 2 min to remove surplus buffer. The samples were applied in 300-μL aliquots, left at room temperature for 5 min and the eluent collected in a tube by centrifugation. The manufacturers state that XAD-2 beads will remove most types of organic molecules. Personal experience has shown the method to be effective with Tween 20 and to involve little, or no, loss of ABH activity from nonblood body fluid samples.[2]

Results

Absorption-elution produced strong agglutination of the indicator cells. The agglutination occurred in case samples treated with anti-A serum. This applied to dilutions up to 1 in 40 of the original stain extracts. The results were those to be expected for a person of group A, except for the lack of agglutination with H lectin.

The attempts to determine the PGM group were unsuccessful, no enzyme activity being detected. This is further evidence that "Tabort" had affected the normal biochemical reactions to be expected from a seminal stain.

The ELISA results are shown in Table 1. The ELISA procedure can lack precision so that to interpret the data it is necessary to take into account the standard deviation of the replicates of each assay. To do this, the optical densities for each sample with each antiserum

[2]S. Bolton and J. W. Thorpe, unpublished observation.

TABLE 1—*ELISA results.*

Sample Number	ABO Group of Donors	Sample		Treatments, Optical Densities at 490 nm (Mean, $n = 4$)			No. Antibody Control
		Tabort Treated	Amberlite Treated	Anti-A	Anti-B	Anti-H	
1	A	yes	no	0.02	0.00	0.00	0.00
2	A	yes	yes	.21	0.00	0.86	0.00
3	A	no	no	1.37	0.01	0.67	0.01
4	A	no	yes	1.46	0.01	0.56	0.01
5	B	yes	no	0.01	0.01	0.01	0.01
6	B	yes	yes	0.04	0.30	0.04	0.01
7	B	no	no	0.01	0.22	0.10	0.00
8	B	no	yes	0.02	0.35	0.05	0.00
9	O	yes	no	0.02	0.01	0.02	0.01
10	O	yes	yes	0.05	0.01	0.97	0.01
11	O	no	no	0.02	0.01	1.15	0.00
12	O	no	yes	0.01	0.01	1.00	0.01
13	no stain	yes	no	0.01	0.02	0.00	0.01
14	no stain	yes	yes	0.01	0.02	0.00	0.01
15	unknown	unknown	no	0.00	0.00	0.00	0.00
16	unknown	unknown	yes	0.51	0.00	0.06	0.00

were scaled by dividing the mean response by the standard deviation of the four replicates [7]. The results are illustrated in Fig. 1.

Discussion

The results clearly show the recovery of information under adverse conditions and also provide a comparison between absorption-elution and ELISA as a means of determining ABO groups of nonblood body fluids.

Both A/E and ELISA gave results. The ELISA procedure showed a susceptibility to de-

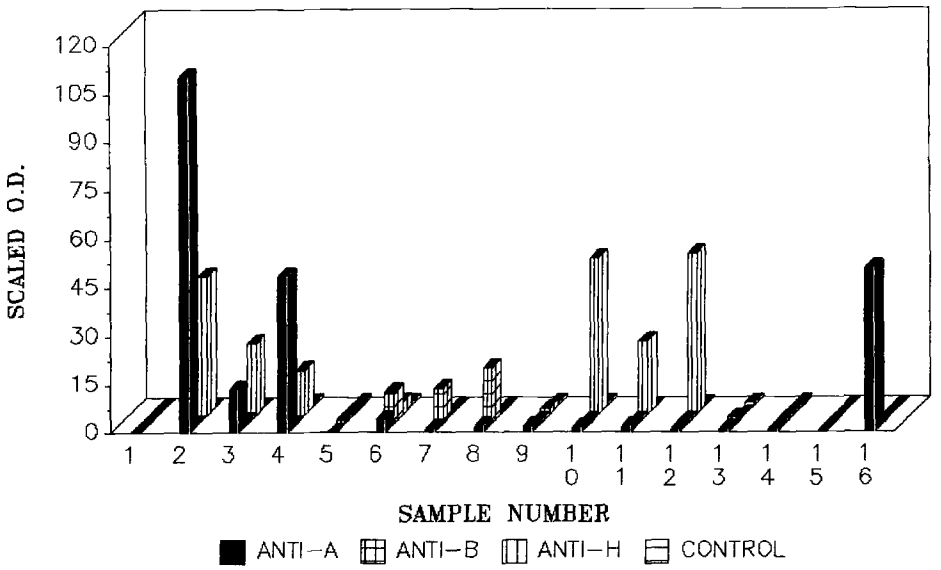

FIG. 1—*Optical densities scaled to the standard deviation for treated and untreated samples.*

tergents but this was overcome using the Amberlite XAD-2. Estimates of the quantities of sample used suggested that there was little difference in sensitivity between the two procedures. The most significant difference in the two procedures was in the ability to detect H substance.

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Address requests for reprints or additional information to
Sara Bolton
20 Penmoel Row (Off Bishton Lane)
Woodcroft, Near Chepstow,
Gloucestershire NP6 7PY United Kingdom