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Evaluation of Monoclonal Anti-A and Anti-B and Affinity-purified *Ulex europaeus* Lectin I for Forensic Blood Grouping*,**

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Summary. Two different monoclonal anti-A and anti-B and several different affinity purified *Ulex europaeus* lectin I reagents were evaluated and compared with conventional anti-A and anti-B sera and *Ulex* anti-H for serologic properties, in inhibition tests with secretor salivas, and in elution tests with bloodstains. The monoclonal and purified reagents were found to be comparable to conventional ones, and accordingly suitable for forensic inhibition and elution procedures.

Key words: Monoclonal antibody – ABO system, bloodstains – Absorption inhibition, bloodstains – Absorption elution, bloodstains

Zusammenfassung. Zwei verschiedene monoklonale Anti-A- und Anti-B-Reagenzien sowie verschiedene Lectin-I-Präparate, durch Affinitätschromatographie aus *Ulex-europaeus*-Samen gereinigt, wurden mit üblichen Anti-A- und Anti-B-Seren sowie *Ulex*-Anti-H hinsichtlich ihrer serologischen Eigenschaften im Hemmtest mit Sekretor-Speichel und im Elutionstest bei Blutspuren miteinander verglichen. Die monoklonalen Reagenzien und *Ulex*-Präparate sind mit den bisher üblichen Reagenzien durchaus vergleichbar und eignen sich gleichermaßen für forensische Hemm- und Elutionstests.

Schlüsselwörter: Blutgruppen, monoklonale Antikörper – Anti-H, *Ulex*-Präparate

The determination of ABH antigens in blood or body fluid stains as a means of partial individualization has been of interest to forensic investigators for decades. A number of different procedures have been proposed and used for this purpose, and many technical modifications and refinements have appeared

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in the literature (Gaensslen 1983). At present, ABH antigens in bloodstains are commonly determined by absorption-elution (Kind 1960), while absorption-inhibition techniques are commonly used to detect the soluble ABH substances characteristic of secretor body fluids.

Until very recently, anti-A and anti-B sera of human origin were the only commonly used reagents of their kind. The finding that hybrid plasmacytoma cell lines which made antibody against preselected antigens could be obtained, however, opened the possibility of preparing monoclonal antibodies to any desired antigen (Köhler and Milstein 1975). The explosive growth of monoclonal antibody technology and its applications have been extensively discussed elsewhere (Kennett 1979; Milstein et al. 1979; Kennett et al. 1980; Zola 1980; International Forum 1983). Of significance in the present context has been the preparation of a number of monoclonal blood grouping antibodies with various specificities (Doinel et al. 1982; Sonneborn et al. 1983; Voak et al. 1983). A number of laboratories have prepared monoclonal anti-A and anti-B, and evaluated them as blood grouping reagents in comparison with conventional polyclonal antisera of human origin (Barnstable et al. 1978; Majdic et al. 1979; Voak et al. 1980; Edelman et al. 1981; Sacks and Lennox 1981; Doinel et al. 1982; Johnson et al. 1982; Rouger et al. 1982; McClelland and McGowan 1983; Sonneborn et al. 1983; Voak et al. 1983).

Several of the monoclonal anti-A and -B preparations were found to have serologic characteristics comparable to those of conventional antisera as cell typing reagents, although avidity was lower in some cases. Accordingly, the monoclonal antibody preparations deserve serious attention as possible replacements for the antisera currently used in blood and body fluid stain grouping. The potential value to forensic serologists of essentially inexhaustible supplies of well defined, structurally homogeneous antibody preparations has been discussed by Fletcher and Davie (1980).

Besides anti-A and anti-B, the anti-H lectin from *Ulex europaeus* seeds (Wiener et al. 1958) has been another important reagent in forensic ABH grouping tests for some time. Anti-H grouping reagent is usually a crude saline or phosphate-buffered saline extract of ground *Ulex* seeds. Preparations of this kind are commercially available as well. Biochemical studies have shown that crude *Ulex* seed extracts contain two lectins, one inhibited by L-fucose and the other by di-N-acetylchitobiose and other oligosaccharides (Matsumoto and Osawa 1969, 1970, 1972; Horejsi and Kocourek 1974; Pereira et al. 1978, 1979). The L-fucose-inhibitable lectin has been called "UEA I" (for "*Ulex europaeus* agglutinin I"). It reacts preferentially with red cells of groups 0 and A_2 in agglutination tests, is inhibited by the soluble H-active oligosaccharides of secretor body fluids in inhibition tests, and represents the "anti-H" activity in crude *Ulex* seed extracts. The other lectin (UEA II) is a human red cell agglutinin, but shows relatively little ABH specificity. Blake et al. (1982) have suggested that UEA I preparations free of UEA II, such as affinity-purified UEA I, might prove to be more sensitive or specific in forensic elution and inhibition tests than the crude *Ulex* seed extracts.

An evaluation of an affinity-purified UEA I recently carried out by Harris-Smith and Fletcher (1983) indicated that the reagent behaved satisfactorily in

inhibition tests with secretor body fluids, but that it gave weak or inactive eluates in elution tests with group 0 bloodstains.

A two-dimensional titration scheme for the evaluation and comparison of inhibition characteristics of different anti-A and anti-B sera with the soluble blood group substances in secretor body fluids was described by Wiener and Kosofsky (1941a, b). Lincoln (1973) and Lincoln and Dodd (1973) described and studied the parameters affecting the elution of blood group antibodies from bloodstains. This work provides the basis for an approach to evaluating and comparing the absorption and elution characteristics of different antisera with bloodstain antigens. Using these methods, we have recently evaluated a large number of commercial antisera for applicability to bloodstain grouping (Gaensslen and Lee 1983). In this paper, we report the evaluation of two examples of monoclonal anti-A and anti-B and several examples of affinity purified UEA I, and their comparison with conventional ABH typing reagents, in inhibition tests with secretor saliva and elution tests with bloodstains.

Materials and Methods

Conventional human anti-A and anti-B (designated "PC" for "polyclonal") were obtained from American Dade through American Scientific Products, Boston, MA, USA; Immucor, Inc., Norcross, GA, USA; and Folex-Biotest-Schleussner, Inc., Fairfield, NJ, USA. Two lots each of monoclonal anti-A and anti-B (designated "MC1" and "MC2") were the generous gift of Folex-Biotest-Schleussner, Inc. Crude anti-H reagent (designated "Cr1") was prepared by the method of Kind (1962) from *Ulex europaeus* seeds obtained from F. W. Schumacher Co. *Ulex* anti-H obtained from Dade was designated "Cr2". UEA I was obtained from Polysciences, Inc., Warrington, PA, USA (Lot 3-2197 designated "UEAI-AP1", Lot 23172 designated "UEAI-AP2") and Miles Laboratories, Elkhart, IN, USA (Lot UF6 designated "UEAI-AP3"). Another example of affinity purified UEA I (designated "UEAI-AP4") was the generous gift of Dr. E.T. Blake, Forensic Science Associates, Emeryville, CA, USA. UEA I was reconstituted in 10 m*M* sodium phosphate-buffered saline, pH 7.4. Stock solutions of all UEA I reagents were 0.625 mg/ml. AB serum was from Dr. Molter GmbH, Heidelberg, FRG, or BCA-Accugenics, Malverne, PA, USA. Low-ionic strength solution (LISS) was prepared according to the method of Löw and Messeter (1974) or obtained from Ortho Diagnostics, Raritan, NJ, USA. Papain was obtained from Sigma Chemical Co., St. Louis, MO, USA, and papain solution was prepared according to the procedure of Löw (1955). Papain treatment of red cells was according to the method of Boorman et al. (1977). Bovine albumin was from Ortho or Dade. Panels of group A_1, A_2, B, and 0 reagent red blood cells were obtained from Immucor.

Titrations were carried out in 6 × 50 mm test tubes, using doubling antiserum or lectin dilutions, 50 µl as one volume, and equal volumes of antisera and 0.1% red cell suspensions. AB serum, LISS, phosphate-buffered saline (PBS) or saline-albumin were used as titrating diluent and as cell suspending media in titrations involving them. AB serum medium is human AB serum diluted 1:10 with saline and albumin medium is saline containing 0.5% bovine serum albumin. PBS is normal saline made in 50 m*M* sodium phosphate buffer, pH 7.2. Tube contents were mixed, incubated at room temperature for 15 min, briefly centrifuged, and transferred to Boerner slides for rotation and microscopic reading. Doubling dilutions (and titers) greater than 512 are denoted 1T, 2T, 4T, 8T, etc. (where "T" stands for "thousand"). These values are equivalent to 1024, 2048, 4096, 8192, etc. Titration scores were based on a scheme modified from Issitt and Issitt (1976): 4+ = 12, 3+ = 10, 2+ = 8, 1+ =5, w = 2, and negative = 0.

Inhibition tests essentially followed the procedure of Wiener and Kosofsky (1941a, b). Freshly collected saliva from secretors of groups A, B, and 0 was diluted 1:1 with saline,

placed in a boiling water bath for 5 min, then centrifuged for 5 min to remove solid matter. Doubling dilutions of saliva were made in tubes, and an equal volume of antiserum or lectin was added. After 4 h at 4°C, a volume of 0.1% test cells was added. Contents of the tubes were mixed, allowed to stand 15 min at room temperature, briefly centrifuged, then transferred to Boerner slides for rotation and microscopic reading. Inhibitive titer is defined as the last tube in the saliva dilution series giving a negative result. Anti-A was tested with A secretor saliva using A_2 test cells, anti-B with B secretor saliva and B cells, and anti-H with 0 secretor saliva and 0 cells.

Elution tests were carried out in 12 × 75 mm test tubes on 1-mm^2 portions of group A_1, B, or 0 bloodstains on cotton cloth approximately 4 months old. Antisera or lectin was diluted, if necessary, to a titer of 256 (in some cases 512), sufficient material was added to cover the stain, and absorption carried out at 4°C for 17 h. Excess antiserum was removed, and the stain was washed six times with 4°C saline by filling the tube to the top and allowing 15 min between washes. Elution was carried out into 110 µl saline for 20 min at 56°C. Eluates were quickly removed and titrated by transferring two 50-µl aliquots to the first two members of a row of 6 × 50 mm test tubes previously prepared for doubling dilution titration, after which the procedure was the same as described for titration. Eluate titers (and titration scores) provide a relative measure of antibody yield from the bloodstain sample. In some experiments, one of the conditions in the absorption or elution stages was systematically varied, while holding all others constant. The elution experiments were carried out using papain technique as an experimental device for enhancing antibody titers.

Results and Discussion

The monoclonal (MC) anti-A and anti-B and UEA I reagents were titrated along with conventional polyclonal (PC) antisera and *Ulex* seed crude extract anti-H under various serologic test conditions. Representative results are shown in Table 1. Both PC and MC anti-A showed enhanced titers and/or scores in AB serum and with papain technique. The effect of LISS was minimal. Titers of PC anti-A are almost always higher with A_1 than with A_2 cells, and the MC anti-A behaved similarly although the difference was often not as great. PC antisera generally showed higher scores than MC reagents, an effect which was more apparent in saline and LISS than in AB serum or with papain technique. PC anti-B sera are not ordinarily enhanced as much in AB serum or with papain-treated cells as corresponding anti-A. The MC anti-B exhibited somewhat higher scores in AB serum and with papain relative to saline than the PC antisera. No significant differences in titer or score were apparent with PC or MC reagents comparing saline, albumin, and PBS media. These results are consistent with those of Doinel et al. (1982), Voak et al. (1980), and Sacks and Lennox (1981) to the extent that they can be compared. The conditions used in the present studies were somewhat different from those of the other studies.

Crude extract *Ulex* seed anti-H typically has a titer in the 64 to 256 range with 0 cells, and one or two dilutions lower with A_2 cells, and shows significant enhancement in AB serum or with papainized cells. Titers with A_1 and B cells vary, but are always lower than those with A_2. Voak et al. (1983) obtained similar results with crude anti-H against 0 cells in comparing saline and AB serum media and papain technique. Different UEA I reagents at the concentration tested showed varying titers with 0 cells. AB serum medium and papainized cells characteristically increase the titer and score relative to saline. In a number of titrations with these reagents, zone effects were observed. Under such

Table 1. Characteristics of antiserum and lectin reagents under different test conditions

Reagent	Test cell phenotype	Titer (score) under indicated serologic test condition			
		Saline	Papain	LISS	AB serum
Anti-A (PC)	A_1	512 (96)	4T (123)	1T (104)	1T (111)
	A_2	256 (82)	1T (99)	256 (82)	512 (96)
Anti-A (PC)	A_1	1T (88)	4T (119)	1T (102)	2T (94)
	A_2	256 (85)	512 (94)	256 (89)	512 (94)
Anti-A (MC1)	A_1	128 (67)	2T (91)	256 (72)	512 (81)
	A_2	128 (56)	256 (76)	128 (62)	128 (62)
Anti-A (MC2)	A_1	256 (75)	1T (99)	256 (75)	512 (103)
	A_2	64 (55)	256 (92)	128 (67)	128 (72)
Anti-B (PC)	B	512 (103)	1T (115)	1T (111)	512 (107)
Anti-B (PC)	B	512 (101)	1T (108)	1T (104)	1T (107)
Anti-B (MC1)	B	256 (75)	512 (97)	256 (78)	1T (91)
Anti-B (MC2)	B	128 (67)	256 (77)	128 (77)	256 (93)
Anti-H (Cr1)[b]	0	128 (77)	512 (89)	NT[a]	512 (93)
Anti-H (Cr1)[b]	0	128 (73)	512 (92)	NT	256 (73)
	A_2	64 (59)	128 (72)	NT	128 (68)
	A_1	2 (10)	8 (30)	NT	16 (28)
	B	8 (29)	64 (63)	NT	16 (36)
UEA I (AP1)	0	128 (42)	8T (72)	NT	32T (Z)[c]
UEA I (AP2)	0	512 (50)	8T (70)	1T (92)	32T (Z)
UEA I (AP3)	0	8T (Z)	128T (Z)	1T (92)	NT
	A_2	16 (12)	512 (53)	NT	NT
	A_1	16 (22)	64 (37)	NT	NT
	B	2T (Z)	8T (145)	1T (Z)	NT
UEA I (AP4)	0	2T (Z)	8T (145)	1T (Z)	NT

[a] NT, not tested
[b] Different examples
[c] (Z), zone effect

circumstances, a meaningful score cannot be obtained. The reactions of UEA I reagents with A_2, A_1, and B cells do not necessarily follow the same pattern that is commonly seen with the crude *Ulex* anti-H.

The inhibition characteristics of the reagents with the soluble A, B, and H substances of secretor saliva were compared by a two-dimensional titration-inhibition scheme. Inhibitive titers are shown in Table 2. The MC anti-A and anti-B were significantly better inhibited than the corresponding PC antisera except at the highest antiserum concentration, and the affinity-purified (AP) UEA I reagents were better inhibited than crude extract anti-H. By this measure, inhibition tests with the MC and AP reagents would be expected to be more sensitive than those with conventional ones, particularly at the lower antiserum and lectin concentration at which inhibition tests are commonly performed. A monoclonal anti-A studied by Edelman et al. (1981) was well inhibited by A secretor saliva, and Voak et al. (1983) noted good inhibition

Table 2. Inhibitive titer determinations

Reagent titer	Inhibitive titer with indicated reagent and corresponding secretor saliva							
	Anti-A (PC)	Anti-A (MC1)	Anti-B (PC)	Anti-B (MC1)	Anti-H (Cr1)	Anti-H (Cr2)	UEA I (AP1)	UEA I (AP2)
128	2	2	2	16	NT[a]	NT	8	4
64	2	16	32	256	NT	NT	32	8
32	8	64	64	1T	0	NT	128	32
16	64	1T	256	2T	1	32	256	128
8	2T	64T	1T	128T	8	64	512	1T
4	NT	NT	NT	NT	64	256	2T	4T

[a] NT, not tested

Table 3. Effect of titer of absorbing antiserum or lectin on eluate antibody yield

Absorption titer of reagent	Titer (score) of eluate from bloodstain of corresponding phenotype using							
	Anti-A (PC)	Anti-A (MC1)	Anti-B (PC)	Anti-B (MC1)	Anti-H (Cr1)	UEA I (AP2)	UEA I (AP3)	UEA I (AP4)
512	NT[a]	NT	NT	NT	16 (35)	128 (69)	64 (58)	32 (55)
256	16 (31)	8 (28)	32 (42)	32 (44)	8 (30)	64 (55)	64 (53)	32 (46)
64	4 (18)	8 (26)	8 (29)	16 (31)	8 (23)	32 (47)	16 (38)	32 (48)
16	1 (7)	2 (15)	4 (33)	4 (20)	4 (18)	8 (33)	4 (20)	16 (38)
4	0 (2)	0 (2)	1 (7)	1 (7)	1 (5)	2 (13)	2 (10)	2 (13)

[a] NT, not tested

Table 4. Effect of absorption time on eluate antibody yield

Absorption time (h)	Titer (score) of eluate from bloodstain of corresponding phenotype using							
	Anti-A (PC)	Anti-A (MC1)	Anti-B (PC)	Anti-B (MC1)	Anti-H (Cr1)	UEA I (AP1)	UEA I (AP2)	UEA I (AP4)
1	1 (7)	0 (2)	0 (2)	0 (2)	4 (17)	0 (2)	2 (10)	2 (10)
4	2 (12)	2 (10)	4 (15)	4 (15)	16 (25)	8 (20)	4 (20)	4 (15)
8	4 (17)	8 (20)	16 (25)	8 (23)	16 (36)	8 (22)	16 (36)	32 (36)
17	16 (30)	16 (33)	32 (33)	16 (31)	32 (38)	16 (28)	64 (49)	64 (51)
34	16 (33)	32 (38)	32 (35)	16 (33)	16 (41)	16 (30)	64 (53)	64 (53)

characteristics for monoclonal anti-A and anti-B with corresponding secretor salivas. The latter group noted that inhibition tests with the anti-B were four dilutions more sensitive with A_1B than with B cells. Harris-Smith and Fletcher (1983) obtained good inhibition results with 0 secretor saliva using an AP UEA I. The inhibitive titers with the AP reagent and the conventional anti-H in their studies were comparable to those obtained in the present work.

Absorption elution characteristics were examined by looking at the effects of absorption titer, absorption time, elution temperature and elution time on the titer of the eluate obtained with bloodstain samples.

The effect of absorption antiserum or lectin titer is seen in Table 3, and that of absorption time in Table 4. Eluate titers increase as absorption antiserum or lectin titer increases over the range examined. These results are consistent with previous findings for PC anti-A and anti-D sera (Lincoln and Dodd 1973; Gaensslen and Lee 1983). The MC antibodies and the AP UEA I reagents exhibit the same behavior as the corresponding PC antibodies and crude *Ulex* anti-H in this respect. Eluate titers also increase with increasing absorption times with saturation occurring at about 17h. This effect is likewise consistent with previous studies of this kind. The MC and AP UEA I show similar kinetics and eluate antibody yields comparable to the PC antisera and crude *Ulex* anti-H.

The effect of elution temperature is shown in Table 5. Elution is most often carried out at 56°C, following Landsteiner and Miller (1925). Previous studies indicated that PC anti-A eluted optimally at 55°–60°C, the antibody yield decreasing again at 65° and 70°C, while anti-D yield increased slightly at 65° and 70°C as compared with 56°C using papain technique (Lincoln and Dodd 1973). Both MC and PC anti-A and anti-B showed optimal antibody yields at 56°C, the yield being lower at 65°C. Eluate anti-H yields increased with all the reagents up to 56°C as well, the results at 65°C being more variable. The fact that some antibody and lectin elute at 26°C and even more at 37°C emphasizes the importance of washing bloodstain samples at 4°C during elution tests.

Eluate antibody and lectin yields were also compared for the different reagents with elution times of 5, 10, 20, and 30min at 56°C. Both MC and PC anti-A and anti-B and AP and crude anti-H behaved similarly in this respect. Yields increased as elution times were lengthened from 5 to 20min. With several reagents, modest additional increases in yield were seen at 30min elution as compared with 20min, but these effects were not exclusive to MC antibodies as compared with PC ones, nor to AP anti-H as compared with crude *Ulex* reagent.

Voak et al. (1983) carried out absorption elution studies on A_1 and A_2 cells with MC anti-A and on B cells with MC anti-B, and obtained good eluate titers. Harris-Smith and Fletcher (1983) compared an AP UEA I reagent with conventional anti-H in absorption elution tests on group 0 bloodstained cloth using the Howard-Martin (1969) technique and on dried ammoniacal extracts of 0 bloodstains (Kind and Cleevely 1969). Eluates obtained with the AP reagent

Table 5. Effect of elution temperature on eluate antibody yield

Elution temperature (°C)	Titer (score) of eluate from bloodstain of corresponding phenotype using								
	Anti-A (PC)	Anti-A (MC1)	Anti-B (PC)	Anti-B (MC2)	Anti-H (Cr1)	UEA I (AP1)	UEA I (AP2)	UEA I (AP3)	UEA I (AP4)
4	0	0	0	0	1 (5)	2 (10)	1 (7)	1 (5)	1 (10)
26	1 (5)	0 (2)	1 (5)	1 (7)	4 (18)	16 (38)	4 (17)	2 (12)	2 (15)
37	4 (18)	2 (10)	1 (7)	4 (20)	4 (20)	32 (46)	8 (25)	4 (20)	8 (23)
56	16 (33)	8 (20)	2 (15)	16 (40)	16 (43)	64 (63)	128 (56)	128 (67)	64 (51)
65	2 (12)	1 (7)	1 (5)	2 (13)	32 (39)	8 (31)	32 (48)	128 (64)	64 (39)

were unreactive or very weak in saline and only slightly improved in 2% or 5% albumin media, while those with the conventional anti-H were strong. The AP UEA I reagents tested in the present studies gave eluates from group 0 bloodstains comparable to those obtained with conventional anti-H using a tube elution method and papain technique. We have obtained comparable eluates from group 0 bloodstains with conventional anti-H and UEA I reagents in saline as well, although the eluate titers were lower.

The results obtained here indicate that MC anti-A and anti-B and AP UEA I reagents tested gave results comparable to those of conventional reagents in inhibition and elution tests, and may be considered suitable for forensic blood and body fluid testing. Absorption elution tests similar to those in the present study have been carried out on dried ammoniacal extracts of bloodstains. The results will be reported elsewhere.

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