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ABO blood grouping of hairs using an Avidin-Biotin-Peroxidase Complex technique

K. Nishi, B. Annuß, S. Rand, and B. Brinkmann

Institut für Rechtsmedizin der Westfälischen Wilhelms-Universität Münster, von-Esmarch-Straße 86, D-4400 Münster, Federal Republic of Germany

Summary. Fifty-nine hair specimens obtained from human autopsies and volunteers were used for the determination of ABO blood group substances using the ABC (Avidin-Biotin Complex) technique. Positive staining for A, B and H blood group substances was detected only in the medulla of the hairs. Blood group antigens could not be detected in seven hair specimens because they possessed no medulla. Forty-seven specimens obtained from fresh cadavers and volunteers gave the correct results corresponding to the blood group of the donor, but some specimens from individuals of blood group A_2, Le(a+b−) showed weak reaction with anti-A and strong reaction with anti-H. The staining intensity with anti-B and -H in some individuals of blood group AB was stronger than with anti-A serum. Five hair specimens obtained from decomposed bodies were also examined. The blood group antigens could be specifically detected in hairs obtained from two exhumed and one putrid body, but no positive reactions were obtained from two cases of drowning where the bodies had been in the sea for about 6 months. In a blind trial, hair specimens from 28 individuals were also examined. Twenty-two specimens which possessed a medulla gave the correct result. Six specimens gave no result because they possessed no medulla.

Key words: ABO grouping on hairs, immunohistochemistry – Avidin-Biotin Complex, ABO grouping on hairs

Zusammenfassung. An 59 menschlichen Haarproben von Leichen und lebenden Personen wurden die ABO-Blutgruppen mittels der Avidin-Biotin-Technik untersucht.

Eine positive Reaktion für A-, B- und H-Blutgruppen-Antigene konnte nur im Markstrang beobachtet werden. Wegen eines fehlenden Markstranges gelang der Nachweis in einigen Proben nicht.

Bei 47 Haarproben von frischen Leichen und Lebenden stimmte das Untersuchungsergebnis mit der festgestellten Blutgruppe überein, aber einige Proben von Personen der Blutgruppe A_2, Le(a+b−) zeigten eine schwache Reaktion mit Anti-A und eine starke mit Anti-H. Die Reaktion mit Anti-B

Offprint requests to: K. Nishi

und Anti-H bei einigen Fällen der Blutgruppe AB war stärker als mit Anti-A-Serum. Auch fünf Haarproben von fäulnisveränderten Leichen wurden untersucht. Bei zwei exhumierten und einer hochgradig fäulnisveränderten Leiche gelang der Blutgruppennachweis sicher, bei zwei Wasserleichen mit einer Liegezeit von 6 Monaten nicht.

Von 28 Haarproben einer Blindstudie konnte in 22 Fällen ein positives Ergebnis erzielt werden, in sechs Fällen wegen des Fehlens eines Markstranges kein Ergebnis.

Schlüsselwörter: ABO-Blutgruppenbestimmung an Haaren, Immunhistochemie – Avidin-Biotin-Komplex, ABO-Blutgruppenbestimmung an Haaren

Introduction

The first successful demonstration of the presence of ABH antigens in human hair was reported by Sakai (1951). Since then, many attempts to identify the ABO blood groups from hairs have been made and many different approaches have been used including absorption-elution, direct agglutination, mixed agglutination, radioimmunoassay and immunofluorescence methods (Krüger and Hummel 1981).

In 1966 Yada et al. introduced an absorption-elution method and since then ABO grouping from hairs has been extensively investigated using this method albeit with some controversies over its reliability, especially in hairs from Europeans (Brinkmann and Lemke 1979; Oepen and Noever 1980).

Recently, indirect immunoperoxidase methods have been used to locate ABH blood group antigens in tissue sections and in 1984 the Japanese groups of Miyasaka et al. and Yoshida and Ono investigated this method and suggested its possible application for the detection of ABH antigens in hair. These investigations were followed by several reports which confirmed the usefulness of this method (Pötsch-Schneider et al. 1986; Miyasaka et al. 1987). The present investigation was carried out to test the reliability and reproducibility of ABH antigen detection in hairs obtained from Europeans using the Avidin-Biotin Complex (ABC).

Materials and methods

Head hair samples were obtained from 52 human autopsy cases and from seven volunteer donors (42 males, 17 females; age range: 2–93 years). Pubic hair, chest hair, eyelashes, moustache and beard hairs were also taken from seven selected donors. Hair samples were also obtained from 28 volunteers (10 males, 18 females; age range: 3–60 years) and tested in the form of a blind trial. All samples were washed successively with a detergent and ethanol, air-dried and then examined microscopically to select the hairs which possessed a medulla.

Antisera and reagents used in this study were purchased from Dakopatts, Denmark (mouse monoclonal anti-A, -B, and -H serum, biotinylated rabbit anti-mouse immunoglobulin, Avidin-Biotin Complex) and Biotest, FRG (mouse monoclonal anti-A and -B serum).

The hairs were sliced longitudinally by hand and cut into small pieces (each approx. 3 mm) before being subjected to the immunohistochemical staining procedure. This was carried out

in glass test-tubes (0.5 × 2.0 cm), three for each sample and each contained two or three pieces of the cut hair. The hair specimens were washed with Tris buffer (0.05 *M* Tris-HCl-buffered saline, pH 7.5) under reduced pressure and treated as follows:

1) Immersion in Tris buffer containing 1% bovine serum albumin at room temperature for 60 min.
2) Addition of mouse monoclonal antiserum (anti-A, -B and -H, 1:20, Dakopatts, or anti-A 1:200, anti-B 1:400, Biotest) overnight at 4°C.
3) Rinsing with Tris buffer 3 times (each 5 min).
4) Addition of biotinylated rabbit anti-mouse immunoglobulin (1:250 Dakopatts) for 60 min at room temperature.
5) Rinsing in Tris buffer 3 times (each 5 min).
6) Addition of AB complex (Dakopatts) for 60 min at room temperature.
7) Rinsing in Tris buffer 3 times (each 5 min).
8) Immersion in 0.1 *M* acetate buffer (pH 5.2) containing 0.03% 3-amino-9-ethylcarbazole (AEC) and 0.003% H_2O_2 solution for 30 min.
9) Rinsing in water and mounting in Gurr (BDH Chemicals, England). The samples were examined microscopically for positive (red) coloration.

Results

The results obtained in this study are listed in Tables 1 and 2a–c and examples of the reactions obtained are shown in Fig. 1 (a, b).

As can be seen, no false positive reactions were obtained thus substantiating the work of previous investigators (Pötsch-Schneider et al. 1986). The 59 specimens included most ABO and Lewis combinations and a variety of hair colours. The positive staining of ABH antigens was recognized microscopically only in the medulla of the hair. Prior to testing, the fragments of hair were examined microscopically and only those which possessed a medulla were used. The frequency of hairs which possessed a medulla varied with individuals and with sample source and seemed to be higher in thick head hair than thin hairs and much higher in pubic hair or moustache hairs.

Endogenous peroxidase in the hairs was not recognized (i.e., no background staining). This finding corresponds to that observed by Miyasaka et al. (1984).

Anti-sera used in this study were purchased from two different sources. When anti-sera designated as diagnostic reagents were used, a dilution of up to 1:50 could be used, but nonspecific reactions were often found. Anti-sera designated for immunohistochemical use showed specific reaction in dilutions of up to 1:20.

Since seven hair specimens possessed no medulla, it was not possible to determine the blood group. Forty-seven specimens from fresh cadavers and volunteers gave the correct results corresponding to the blood group of the donors but those hairs from individuals of blood group A_2 showed weaker reactions with anti-A than those from blood group A_1. The hairs from two individuals of blood group A_2, Le(a+b−) and A_2B, Le(a+b−) gave only a weak reaction with anti-A. However, these specimens possessed strong reactivity with anti-H and/or anti-B anti-serum. Staining intensity by anti-B and -H-serum in some individuals of blood group AB was stronger than that by anti-A serum.

Hair specimens from five decomposed bodies (i.e., two exhumed, two drowned and one putrified body) were also included in this study. Although

Table 1. Results of ABO grouping from hairs obtained from fresh cadavers and volunteers using the ABC-technique

Blood group	$N = 47$	Strong reaction	Weak reaction
A_1	$n = 15$		
Le(a−b+)	8	8	0
Le(a+b−)	1	1	0
Le(a−b−)	2	2	0
Unknown	4	4	0
A_2	$n = 5$		
Le(a−b+)	2	2	0
Le(a+b−)	3	1	2[a]
B	$n = 6$		
Le(a−b+)	4	4	0
Le(a−b−)	1	1	0
Unknown	1	1	0
O	$n = 15$		
Le(a−b+)	11	11	0
Le(a+b−)	2	2	0
Le(a−b−)	1	1	0
Unknown	1	1	0
A_1B	$n = 5$		
Le(a−b+)	3	2	1[a]
Le(a−b−)	1	1	0
Unknown	1	1	0
A_2B	$n = 1$		
Le(a+b−)	1	0	1[a]

[a] These specimens showed weak reactivity with anti-A serum and strong reactivity with anti-H and/or -B serum

detection of blood group antigens was possible in two exhumation cases and one putrid one in which the postmortem intervals were 3 months, 4 months and 6 months, respectively, no positive reactions were recognized in the two cases of drowning where the bodies had been in the sea for about 6 months.

In the blind trial hair specimens from six individuals had a fragmented medulla, ten had a broken-line medulla, six had a continuous medulla and six had no medulla. Although the correct results could be obtained from all the 22 specimens which possessed a medulla, the six specimens which possessed no medulla gave no result. Several samples from differing blood groups were found which showed a less than 100% reaction in the medulla, some areas being completely negative (Table 3).

Table 2a. Distribution of blood groups

Blood	Number	Correct	False	Weak
a−b+	28	27	0	1
a+b−	7	4	0	3
a−b−	5	5	0	0
Unknown	7	7	0	0
Total	47	43	0	4

Table 2b. ABO grouping of hairs: avidin-biotin

Blood	Number	Correct	False	Weak
A_1	15	15	0	0
A_2	5	3	0	2
B	6	6	0	0
O	15	15	0	0
A_1B	5	4	0	1
A_2B	1	0	0	1
Total	47	43	0	4

Table 2c. ABO grouping of hairs: blind trial

Blood	Number	Correct	False
A_1	10	9	0
A_2	2	1	0
B	1	1	0
O	15	11	0
Total	28	22	0

Six samples possessed no medulla

Discussion

It seems that there is an obvious difference between Asians and Europeans in the frequency of head hairs which possess a medulla and those which do not. Also, the hair of Asian people is generally thicker than that of Europeans. In this study, it was observed that thicker hairs from Europeans possessed a medulla with relatively higher frequency than thinner hairs, but with a lower frequency than that found in Asians. Pubic hair and moustache hairs, even in European people, show a much higher medulla frequency. It seems from the results obtained in this study that the ABH blood group antigens in hairs are chiefly localized in the medulla. Lincoln and Dodd (1968) reported that 17 examples of hair shavings were grouped correctly, but only 19 of 45 samples of head hair corresponded to the blood group of the donor. It seems likely that their results were dependent on the presence or absence of a medulla in the hairs rather than blood group antigens.

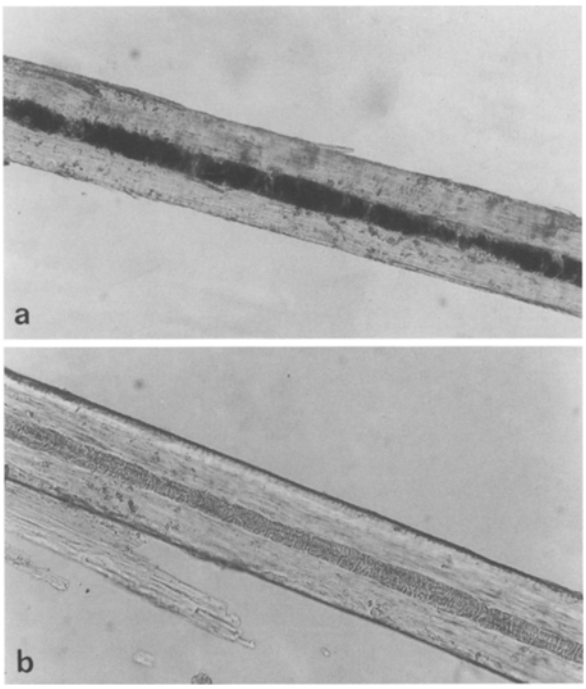

Fig. 1. Results of ABO grouping of hair using ABC staining: **a** positive staining – blood group A_1 against anti-A, **b** negative staining – blood group A_1 against anti-B

Table 3. Results of ABO grouping in a blind trial

Blood group	$N = 28$	Medulla in the hair		Correct result	No result
		present	absent		
A_1	$n = 10$	9	1	9	1
A_2	$n = 2$	1	1	1	1
B	$n = 1$	1	0	1	0
O	$n = 15$	11	4	11	4
Total	$N = 28$	22	6	22	6

In this study, blood group-positive reactions were microscopically recognized only in the medulla. This result shows a gross shortcoming for the forensic practice because blood group determination of hair without medulla would be impossible using the immunohistochemical method. However, in 1986 the Japanese workers, Miyasaka et al. examined the presence of blood group substances in the cortical part of hair using an immuno-electron-microscopical method and have suggested that blood group antigenicity is also located on the inner wall of the vacuole of medulla cells and in the interfibrillar matrix of cortical cells.

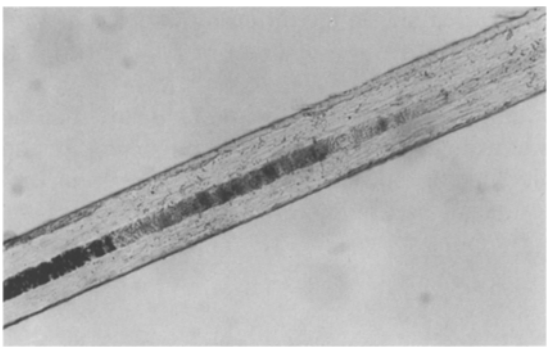

Fig. 2. Example of mosaic staining of the medulla of the hair: blood group A_1 against anti-A

It is well known that ABH blood group substances are extremely stable on storage. Yada et al. (1968) detected blood group antigens from an 88-year-old hair rope. On the other hand, it is also known that blood group antigenicity is destroyed and/or disturbed by many organisms and physicochemical treatments (Pereira and Martin 1976; Mukoyama and Miyasaka 1983; Nagano et al. 1976). In this study, the blood groups of two corpses which had been in the sea for about 6 months could not be determined from the hair samples. The bodies were identified by autopsy and their blood groups were known to be group 0. Although there were no noticeable microscopic changes in the structure of these hairs, the medulla of the hairs showed no reactivity with anti-H serum. This result reveals the necessity to use not only anti-A and -B serum but also anti-H serum for the blood group determination. Ohora et al. (1983) also demonstrated that blood group determination from hairs stored in water or sea water becomes more difficult with time.

The hairs examined in this study included a variety of colours and most ABO and Lewis blood groups. AEC (3-amino-9-ethylcarbazole) allowed easy identification of positive reaction when compared to DAB (diaminobenzidine) even in a sample of black hair. Miyasaka et al. (1984) reported that the bleaching of black hair samples by H_2O_2 was necessary for the detection of the reaction by DAB (dark brown coloration), but can affect blood group antigenicity.

Although all the specimens tested in this study, even in the blind trial, showed the result corresponding to the donor, the hairs from some individuals of blood group A_2 gave a weaker reaction with anti-A serum than those from blood group A_1. This tendency was particularly noticeable in the hairs from two samples of an individual of blood group A_2, Le(a+b−) and from an individual of A_2B, Le(a+b−). These three specimens, however, showed strong reactivity with anti-H and/or anti-B serum. No reasonable explanation for this weak reaction could be found. According to Kishi and Iseki (1977, 1978) ABH blood group substances in hair are carried by glycolipids which are produced independent of the secretor gene in the same way as erythrocyte antigens.

Boettcher and Kay (1973) reported that the presence of group specific substances in hairs was independent of the secretor status. It seems likely that the

weak reactions observed in this study depend on the different amounts of antigenic substances in the hair specimen from the individual of blood group A_2 type, but not on the secretor status.

The mosaic pattern of distribution of staining (Fig. 2) could indicate that the antigens are unevenly distributed throughout the medulla or this phenomenon could be an artifact due to the blocking of the sites by trapped air or to incomplete exposure of the antigen sites during sectioning.

References

Boettcher B, Kay DJ (1973) ABO blood grouping of human hair using radioactively labelled antibodies. Vox Sang 25:420–425

Brinkmann B, Lemke J (1979) Der Einfluß verschiedener Variabler auf den Blutgruppennachweis aus Haaren mit Hilfe der Absorptions-Elutionstechnik. Arch Kriminol 164:93–100

Kishi K, Iseki S (1977) Immunochemical studies on blood group A substance from human hair. Z Rechtsmed 80:191–195

Kishi K, Iseki S (1978) Immunochemical studies on blood group H and B substances from human hair. Z Rechtsmed 82:153–155

Krüger H-J, Hummel K (1981) Attempts at typing ABO blood groups in individual human hairs of less than 4 cm length. Forensic Sci Int 18:253

Lincoln PJ, Dodd BE (1968) Mixed agglutination as a method for the determination of A, B and H blood groups of hair. Med Sci Law 8:38–40

Miyasaka S, Yoshino M, Sato H, Mukoyama H, Seta S (1984) Immunohistochemical investigation on the ABO blood grouping of a minute sample from human scalp hair. Rep Natl Res Inst Police Sci 37:27–35

Miyasaka S, Yoshino M, Sato H, Mukoyama H, Seta S (1986) Identification of the blood group substances in hair by immunoelectronmicroscopical method. In: Proceedings of the 70th Annual Meeting of the Medico-Legal Society of Japan, p 66

Miyasaka S, Yoshino M, Sato H, Miyake B, Seta S (1987) The ABO blood grouping of a minute hair sample by the immunohistochemical technique. Forensic Sci Int 34:85–98

Mukoyama H, Miyasaka S (1983) Effect of fixative reagents on the detection of ABH-antigens from human blood stains and tissues. Rep Natl Res Inst Police Sci 36:137–146

Nagano T, Tsuji T, Ieda K (1976) Blood group determination of severely charred bodies. The effect of heating on the blood activity of A, B, AB and O(H) active glycolipid and A and B active glycoproteins. J Forensic Sci Soc 16:163–168

Oepen I, Noever H (1980) Zur ABO-Blutgruppenprägung des menschlichen Haares. Z Rechtsmed 85:205–209

Ohora Y, Nishimukai H, Inoue K, Hiraoka T, Sakata K, Komura S (1983) Identification of ABO blood group from human hairs in various storage conditions. In: Proceedings of the 67th Annual Meeting of the Medico Legal Society of Japan, p 132

Pereira M, Martin PD (1976) Problems involved in the grouping of saliva, semen and other body fluids. J Forensic Sci Soc 16:151–154

Pötsch-Schneider L, Penzes L, Lorenz K (1986) Immunenzymatische ABO-Blutgruppenbestimmung am Einzelhaar. Z Rechtsmed 97:251–258

Sakai T (1951) Studies on blood group substances of human hairs. Jpn J Legal Med 5:19–41

Yada S, Okane M, Sano Y (1966) Blood grouping of a single human hair by means of elution technique. Acta Criminol Jpn 32:7–8

Yada S, Ishimoto G, Okane M (1968) Blood grouping of an 88-year-old hair rope. Acta Criminol Jpn 34:90–92

Yoshida H, Ono M (1984) Application of immunohistochemical staining to the determination of the ABO blood groups of short gray hair. Rep Natl Res Inst Police Sci 37:8–12

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