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The Detection of A and B Antigens on Human Hair by the Absorption-Elution Technique Using LISS and Papain-Treated Test Cells

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Summary. The absorption-elution technique with low ionic strength solution (LISS) and papain-treated test cells previously used for bloodstains was employed for the detection of AB0 antigens on human hair. Antigen identification was always possible, with good intensity of agglutination, even in those cases where classic techniques had given false-negative results. It was possible to obtain positive results with fragments of human hair as small as 0.2 cm.

Key words: Hair, AB0 detection - Blood group, detection of AB0 on human hair

Zusammenfassung. Die schon für Blutproben verwendete Absorbtions-Elutionsmethode mit LISS (Lösung mit schwach wirkender Ionenstärke) und Testerythrozytensensibilisierung mit Papain wurde von den Verfassern zur Identifizierung der AB0-Antigene in menschlichen Haaren angewandt. Die Identifizierung der Antigene gelang immer mit einer guten Agglutinationsintensität auch in solchen Fällen, in denen die herkömmliche Methodik zu einer falschen Negativität geführt hatte. Die Mindestlänge der Haare für die positive Reaktion war 0,2 cm.

Schlüsselwörter: Haare, AB0-Nachweis - Blutgruppennachweis, AB0 in Haaren

Routine detection of AB0 antigens on human hair started with the first positive experiences of Murakami and Shimizu [1] in 1964, Akaishi [2] in 1965, of Lincoln and Dodd [3] in 1966, who used the mixed agglutination method, and of Yada et al. [4] in 1966 with the absorption-elution method. However, despite continuous improvements in identification techniques, as well as for biologic traces in general, it is known that false-negatives can be obtained. This fact has been confirmed by studies carried out in our laboratory using both the absorption-elution technique and the mixed agglutination procedure [5–7].

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Interesting results have recently been reported by Brinkmann and Lemke [8], who used the absorption-elution method under particular experimental conditions, with bromelain-treated red cells, and obtained neither false-negatives nor false-positives.

The observation that ionic strength influences the antigen-antibody reaction led to the demonstration that a great increase in antibody uptake was obtained, lowering the ionic strength of the reaction medium [9–11].

Lincoln and Dodd [12,13] thus improved the absorption-elution technique, obtaining very good results in antigen identification in bloodstains.

Encouraged by our results o bloodstains, we applied this technique to the examination of human hair by introducing low ionic strength solution (LISS) in various stages of the absorption-elution technique and treating red cells with papain.

Materials and Methods

The LISS composition is described by Low and Messeter [11]:

A) NaCl 0.17 *M*	180
B) Phosphate buffer 0.15 *M* pH 6.7	20
C) Sodium glycinate 0.3 *M* pH 6.7	800

The technique of antigen identification is as follows:

- Hair crushing
- Division into small fragments (0.5 cm)
- Overnight incubation in test tubes with anti-A and anti-B sera (title 1:128–1:256) diluted 1:6 in LISS at +4° C
- Washing with cold saline solution
- Elution in a water bath at 56° C for 15 min
- Addition of one drop of papain-treated A and B test cells in a 0.7% suspension in LISS
- Incubation at +4° C for 1 h
- Centrifugation at 1000 *g* for 1 min
- Macroscopic reading

The red cells were treated with a 1% papain solution, as described by Løw [14] and modified by Jørgensen [15]. Treated red cells were used in a 0.7% suspension in LISS.

We examined hairs from 12 subjects from groups A and B for whom negative results had been obtained with absorption-elution in saline (0.15 *M*). We carried out several studies with different hair specimens from each subject. These results were compared with those obtained using only LISS instead of saline and with those using only papain-treated red cells.

We then examined hair specimens that had previously given positive results with the classic absorption-elution technique, following their reduction in size so that they gave negative results.

Results

The results of the first phase of this study are reported in Table 1, which shows that the number of false-negatives is reduced using LISS instead of saline, and still more with papain-treatment of test cells only. False-negatives are not recorded when LISS and treated red cells are used together.

Table 1. Detection of A and B antigens on human hairs with absorption-elution method: (a) with saline, (b) with only LISS, (c) with only papain-treatment, (d) with LISS and papain-treatment

Case	Group	Saline		LISS				Papain				LISS and papain				*n*
		Pos.	Neg.	–	+	++	+++	–	+	++	+++	–	+	++	+++	
1	A	0	20	9	8	3	0	3	8	9	0	0	4	6	10	20
2	A	0	18	10	5	3	0	7	9	2	0	0	2	7	9	18
3	A	0	14	8	5	1	0	6	7	1	0	0	1	8	5	14
4	A	0	14	10	3	1	0	6	6	2	0	0	3	8	3	14
5	A	0	10	8	2	0	0	6	3	1	0	0	1	4	5	10
6	A	0	12	12	0	0	0	8	3	1	0	0	2	6	4	12
7	A	0	10	8	2	0	0	6	4	0	0	0	1	5	4	10
8	A	0	10	7	3	0	0	5	5	0	0	0	1	6	3	10
9	B	0	14	10	3	1	0	2	4	8	0	0	1	4	9	14
10	B	0	14	8	6	0	0	2	5	7	0	0	3	6	5	14
11	B	0	12	10	2	0	0	2	8	2	0	0	2	5	5	12
12	B	0	15	10	5	0	0	7	8	0	0	0	5	6	4	15
n		0	163	110	44	9	0	60	70	33	0	0	26	71	66	163

Table 2. Comparison of the results obtained with different hair dimensions, with and without the use of LISS and papain treatment

Case	Group	Saline				LISS and papain			
		(length in cm)				(length in cm)			
		0.2	0.3	0.5	2×0.5	0.2	0.3	0.5	2×0.5
1	A	−	−	++	−	−	+	++	++
2	A	−	++	+++	+	+	++	+++	+++
3	A	−	−	++	++	+	+++	+++	+++
4	A	−	−	++	++	++	++	++	++
5	B	−	+	+++	+++	+	++	+++	+++
6	B	−	+	+++	++	++	+++	+++	++
7	B	−	−	++	++	−	++	+++	+++
8	B	−	+	+++	++	+	+	+++	+++

In addition, there is stronger agglutination and there are no false-positives with this method. The sensitivity of the technique was confirmed in the second phase of the study (Table 2).

With the classic absorption-elution technique, the smallest specimen of hair fragments capable of giving positive results was 0.5 cm, while with LISS and papain-treated red cells it was possible to identify the antigens with good agglutination intensity in specimens as small as 0.2 cm.

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