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Problems in Determining A_2B Group Specific Properties in Blood Stains

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Summary. Blood stains belonging to various sub-group A combinations were examined by the absorption-elution method (in comparison with Holzer method and mixed agglutination). No difficulties were encountered in the identification of A_1, A_2, and A_1B blood stains, while identification of A antigen in A_2B stains was dubious (always negative with the others methods). This seemed to be due to low anti-sera titer in relation to A antigen in this subgroup combination.

Zusammenfassung. Mit Hilfe der Absorptions-Elutionsmethode wurden Blutspuren der verschiedenen A-Untergruppenkombination untersucht. Keine Schwierigkeiten traten bei Blutspuren, die A_1, A_2 und A_1B enthielten, auf. Dagegen stieß man auf Schwierigkeiten bei der A-Antigenidentifikation in A_2B-Blutspuren. Wahrscheinlich ist dies auf den niedrigen Titer der Antisera dem A-Antigen dieser Untergruppenkombination gegenüber zurückzuführen.

Key words. Blood groups, A_2B – Blood stains, determining A_2B-group

It is known that the titration of an anti-A serum against red cells of sub-groups A_1 and A_2 shows decreasing values in the order $A_1 > A_1B > A_2 > A_2B$ (Boorman and Dodd). It has also been reported that identification of group specific antigens in blood stains by means of the absorption-elution method may give negative results when the anti-serum titer is low in relation to the antigen concerned. (Fiori, Marigo and Benciolini). On the basis of these considerations, we tried to verify if the reported variation in the anti-A titer of different combinations could present difficulty in the identification of A antigen in blood stains.

Experimental Studies

The following points were investigated:
1. Titration of anti-A and anti-B sera.
2. Identification of A_1, A_2, and A_1B blood stains.
3. Identification of A_2B blood stains.

Identification was carried out employing absorption-elution method, in our experience (Fiori and Coll.) the most specific and sensitive method, according to the forensic exigences. However, experiments were also performed with absorption-inhibition (Holzer test) and mixed agglutination (both in the original Coombs and Dodd procedure and in the recent MCAR test according to Ishiyama and Okada).

1. Titration of Anti-A and Anti-B Sera

In a preliminary study, the titer of anti-sera which were commercially available was determined. These anti-sera are routinely used in our laboratory for blood-group typing. Whereas no significant variation was observed for B antigen (titer range from 1/128 – 1/256) the titer for A-subgroups was A_1 = 1/128, A_1B = 1/64, A_2 = 1/32, A_2B = 1/16.

2. Identification of A_1, A_2, A_1B Blood Stains

Blood stains were prepared on white cotton. For every test, a fragment of stained cotton, corresponding to 1 mg. dry blood, was used. Testing was carried out over a time interval varying from 5 days to 5 months. Stains not older than 7 days were fixed in methanol. Older stains were left unfixed since we observed that desiccation due to aging prevails over the advantages of fixation.

In all the tests carried out, identification of A antigen was always possible with clear agglutination, both with absorption-elution and mixed agglutination tests (lower sensitivity appeared employing Holzer method). Significant variations which could be related to different sub-group combinations were not observed.

3. Identification of A_2B Blood Stains

Blood stains were prepared as described using blood samples from 11 subjects belonging to group A_2B. All stains were analyzed after 5 days and after 50 days, and testing was carried out contemporaneously on fixed (methanol) and non-fixed stains.

Results obtained with absorption-elution method are shown in Tables 1 and 2. These results, which were obtained under different experimental conditions, confirm that it is best to limit fixation to recent stains. Secondly, even under the best experimental conditions, agglutination density for A_2 antigen is not only inferior than that for B antigen, but it is frequently too weak to be useful for identification purposes. In this regard, results in case no. 11 are particularly significant: there was very weak agglutination employing fresh stains and no agglutination using old stains. These reactions prompted further study of the subject's blood type. Results indicated an A_3B subgroup[1].

Negative (or dubious) results were observed employing mixed – agglutination. No significant reduction in titre of the original antiserum were registred with inhibition technique.

Comments

The titration of commercial anti-sera which are in routine use in our laboratory has confirmed the existence of a titer variation within the various sub-group combinations, as reported by Boorman and Dodd.

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[1] *Tests performed.* Anti-A serum = + (controls: against A_1 and A_2 erythrocytes = C); anti-A serum titer = 1/4;
absorbed anti-A serum = negative (controls: A_1 = +++, A_2 = neg.); anti-H serum = + (controls: A_1 +++, A_2 +);
anti-A_1 lectine = neg.; anti-A_2 lectine = +
anti-A_{hel} lectine (Biotest) = late positivity

Table 1. A_2B Bloodstains five days old

Sample N.	anti-A		anti-B	
	Fixed	Unfixed	Fixed	Unfixed
1	++	+	c	+++
2	++	+	c	c
3	++	+	c	+++
4	++	++	c	c
5	+++	+	c	++
6	+	++	+++	++
7	+	±	c	++
8	±	+	c	++
9	++	+ :	+++	++
10	+	++	+++	++
11	(±)	(±)	c	++

Table 2. A_2B Bloodstains five months old

Sample N.	anti-A		anti-B	
	Fixed	Unifixed	Fixed	Unifixed
1	±	±	++	c
2	(±)	+±	+++	c
3	+±	++	++±	+++
4	++	±	+++	c
5	±	±	c	+±
6	±	++	++	+++
7	(±)	++	+++	+++
8	(±)	±	+++	c
9	–	(±)	+++	c
10	–	(±)	c	c
11	–	–	+++	c

These variations do not affect the routine applications of current methods for antigen identification in the A_1, A_2 and A_1B combinations. According to the forensic exigences, absorption-elution appears the most reliable.

On the contrary, the results of our study indicate the A_2B blood group stains give weak positive and at time false negative responses for A antigen, even if absorption-elution is employed.

In our opinion this phenomenon depends on the low anti-A titer as shown especially in case no. 11, where it was only 1/4. On the other hand the reaction of anti-B serum with the same stains gave always strong positive results.

These considerations may explain some false negative results we observed during routine application of the absorption-elution method. These negative results almost exclusively concerned A antigen.

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