Immunochemical detection of ABH antigens in hairs

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INTRODUCTION

The development of new techniques in one field of science has always lead to a proliferation of applications in vastly different areas of research and applied science. This is especially noticeable where the previously used methods have proved to be unreliable or inadequate for the job.

It is not surprising therefore, that the immunohistochemical method first reported in 1979 by Sternberger for the identification of spirochaetes has since been modified and been extensively applied to problems in forensic science. In particular it seems to have found an application in the problem of the detection of A, B and H antigens in hairs.

Since the first recorded reports from Sakai in 1951 and later from the many reports from Yada and his colleagues, the presence of A, B and H antigens in hairs has been extensively studied and the results discussed in depth.

The 1oo % success rate claimed by the japanese workers has never been clearly demonstrated by any of the numerous other groups of workers. Methods ranging from the Absorption-Elution method used by Yada, mixed agglutination by Lincoln (1968), radio-active labelled antibodies by Boettcher (1973) and many others have been employed, but have always produced a quota of false results especially with respect to blood groups A and O.

The first application of the immunohistochemical method was successfully carried out, again in Japan by Miyasaka (1984 and 1987) and also by Yoshida (1984). This was followed by the report from Pötsch-Schneider (1986) who reported correctly determining the blood group of hair samples from 168 individuals using the PAP and APAAP techniques. Specific staining was demonstrated in the medulla of hairs and was found to be independant of secretor status.
In view of previous claims of success this study was carried out to see if the results could be successfully reproduced.

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MATERIALS AND METHODS

The test sample consisted of 59 post-mortem hair samples from individuals ranging from 5-60 years old and post-mortem intervals ranging from 1 day to 6 months. In addition a blind trial of hairs from 28 volunteers was carried out. The technique used was similar to that employed by Pötsch-Schneider except that A, B and H monoclonal antibodies in conjunction with biotinilated rabbit anti-mouse antibody and the sensitive Avidin-biotin-peroxidase complex was used. The hairs were longitudinally sectioned by hand before being treated as described and finally embedded and examined microscopically.

RESULTS AND DISCUSSION

The results are listed in tables 1 (a-c) and as can be seen no false positive results were found, thus substantiating the claim made by the previous investigators. Figures 1,2 show typical positive and negative results obtained during this study.

However the following precautionary comments should be made.

1. As confirmed by all the studies, the antigens were specifically demonstrated but only in the medulla of the hairs. The presence of a medulla varies between races, and can also show inter- and intra-individual variation. The form can range from complete absence through intermittent, broken to continuous so that the necessity of first locating the medulla, or hairs which possess a medulla, must be strongly stressed.
2. The presence of a medulla does not necessarily guarantee a 100 % reaction: several samples were found during the study which showed a mosaic pattern of distribution of staining (fig. 3). The method of longitudinal sectioning would seem to most minimise the risk but when cross-sections are examined care must be taken not to concentrate on too small an area.
3. Completely negative results were found but only in two cases where the body had been in sea water for 6 months. It does however demonstrate the necessity to test for all three A, B and H antigens as is the practice in all other forensic applications of the ABO system.

SUMMARY

A, B and H antigens have been demonstrated with complete success in three independant studies using immunohistochemical techniques and would appear to have a practical application in forensic science. In view of other previous claims of success many more series of tests must obviously be carried out to demonstrate not only the accuracy but also the reproduceability of the results

in the hands of other groups.
One obvious drawback is the wide variation in the presence and type of medulla in caucasians. A microscopical examination of the questioned hair must therefore precede any blood grouping investigation using this method.
The location of A, B and H antigens only in the medulla could explain the discrepancy between previous inconclusive studies where the results obtained were possibly dependant on the presence or absence of a medulla and not on the blood group of the individual.

The observed mosaic distribution of the antigens within the medulla and independance of secretor status can also be observed in other organs and tissue structures of the body. Several theories have been put forward as to their origin and genetic development and it remains to be seen whether the studies on hairs will throw any light onto the now seemingly complicated distribution of the A, B, H and lewis antigens in the human body.

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 1a. ABO grouping results of 59 post-mortem hair samples using the Avidin-Biotin technique. 12 samples were not tested because they possessed no medulla.

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 1b. ABO grouping results from Table 1a in comparison to the distribution of Lewis groups in the sample.

ABO Grouping of Hairs: Blind Trial

	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

6 samples possessed no medulla

Table 1c. ABO grouping results of 28 hair samples in a blind trial. 6 samples were not tested because they possessed no medulla.

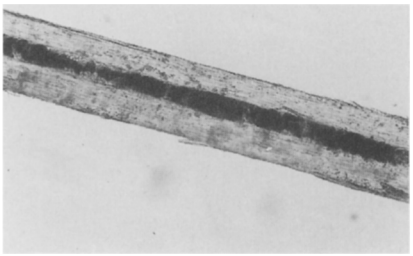

Fig. 1. Positive staining of medulla in a hair from group A_1 individual tested with anti-A.

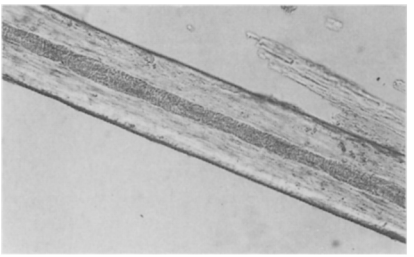

Fig. 2. Negative staining of medulla in a hair from group A_1 individual tested with anti-B.

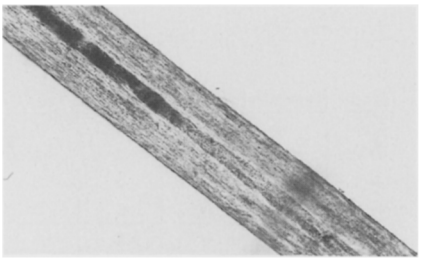

Fig. 3. Mosaic staining pattern of medulla in hair from group A_1 individual tested with anti-A.

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