IMMUNOHISTOCHEMICAL DETECTION OF ABH-ANTIGENS IN HUMAN SALIVA

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The detection of ABH antigens in bodyfluids and subsequent interpretation of results has four main drawbacks:
The amount of sample is often limited: The results are dependant on secretor status: In casework, body fluids are often found in combination with other body fluids: The possible presence of artifacts due to bacterial contamination.

In many cases the translation of ABO results obtained can only be made by assumption especially when one blood group masks another.

In principle, the use of an immunohistochemical technique allows identification of a particular antigen from this mixture and an association to a known morphological structure. For example cells from a group A person could be distinguished from cells from a group O person.

The 3-stage-peroxidase-anti-peroxidase technique (PAP) has been used extensively especially in clinical pathology for identification of cellular antigens and recently Pedal reported successful identification of ABH antigens from paraffin sections of decomposed kidney (1985) and placental tissue (1985).

If the method could be used to identify cellular ABH antigens from a mixture, it would be of immense value in cases of sexual assault.

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As the first stage in this investigation, buccal cells were used as they are readily available from donors, and are essentially the same as vaginal cells. Buccal cells from mouth swabs, were collected from 50 donors on sterile cotton wool swabs and smears made on glass microscope slides. Blood samples were also collected and typed in the ABO and Lewis systems. After drying, the slides were fixed by carefully passing over a flame.

The 3-stage PAP method for the identification of the ABO antigens was carried out as follows (fig. 1):

1. Monoclonal mouse anti-A, -B and Ulex anti-H.
2. Rabbit anti-mouse IgM.
3. Swine anti-rabbit immunoglobin.
4. PAP complex from rabbit.

Visualisation of the PAP complex was performed using hydrogen peroxide and 3-amino-9-Ethylcarbazole resulting in a red colouration for a positive reaction.

Examination of the samples was carried out blind and independantly compared to the ABO and Lewis grouping. In recent experiments we have looked at other types of cells and mixtures of cells of different blood groups. A preliminary report of the findings is included.

Results

No false results were obtained, i.e. the results from PAP typing corresponded to the ABO blood type in each case. No negative results were obtained although some donors were non-secretors, from the Lewis type, suggesting an independance from secretor status (fig. 2).

Some samples showed a weak red colouration but only as a background staining. The cause for this is not clear but so long as only a red staining on the cell membrane was counted as positive no errors were made.

Since the reaction is specific for A, B and H terminal sugars and as bacteria are known to possess similar structures this could be a reason for this phenomenon. If this is so, then the PAP method could distinguish between ABO antigens from cells and any false positives from bacterial contamination, assuming that bacteria have not absorbed onto the cell membrane.

After the success of this survey we have made limited investigations on vaginals swabs with encouraging results.
Here again background staining was observed and only when the cells were stained should a positive reaction be recorded.

Experiments on buccal cells taken from smoked cigarettes have also shown some success.
Our first experiments using spermatozoa only yielded limited success but later attempts after freezing and thawing of samples gave encouraging results. It was noticed that sometimes only approximately 50 percent of cells were stained in the positive sample which might reflect the heterozygote condition.

In conclusion we may state that the situation at present is as follows:

1. The immunohistochemical technique for detection of ABH antigens is a reliable technique.

2. It would seem to have an advantage over serological methods since the reaction can be directly attributed to cellular structures.

3. ABH detection is apparently independent of secretor status.

4. It seems to be possible to discriminate between 2 different cell populations of different ABO types.

5. Identification can be made from very small numbers of cells.

6. Lastly, definite results have been obtained from buccal and vaginal cells and recently also encouraging results from spermatozoa have been obtained.

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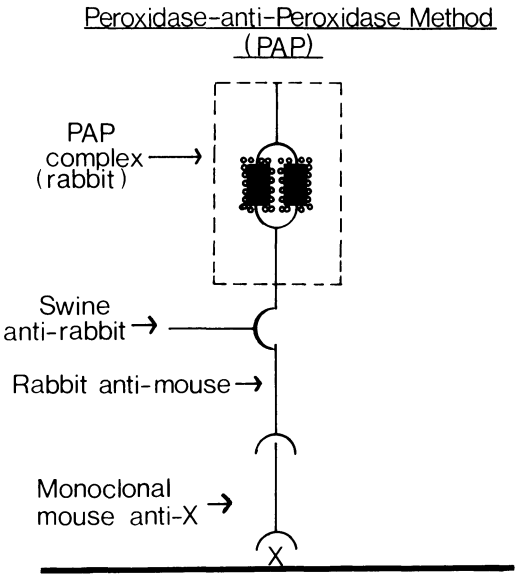

Fig. 1: Diagrammatic representation of the PAP-method

PAP blind trial n=50

blood	n	%	correct
A_1	15	30	15
A_2	4	8	4
B	7	14	7
O	23	46	23
AB	1	2	1

PAP blind trial - Lewis

blood	n	strong +	weak +
a-b+	37	37	-
a+b-	7	2	5
a-b-	4	4	-
unknown	2	2	-

Fig. 2: ABO and Lewis results from blind trial