Determination of ABH antigens in fingernails using the APAAP (immunoalkaline phosphatase) technique

R.Wegener and U.Bulnheim

Doz.Dr.R.Wegener, Institut für Gerichtliche Medizin, Bereich Medizin, Wilhelm-Pieck-Universität Rostock, Friedrich-Engels-Strasse 108, 2500 Rostock 1, DDR

In contrast to many investigations into the presence of ABH antigens in human hairs the reports on the blood grouping of fingernails are rare (YADA et al.1966; KIRST et al.1971). By means of absorption/elution technique the presence of blood group substances was demonstrated without attaching to known morphologic structures of layer of nail. Fragments of fingernails may become traces themselves or stain carrier in forensic cases. Therefore, the direct visualisation of ABH pattern in nail-keratin and the discrimination of adherent blood group substances will be important.

The introduction of immunoenzyme methods in different modifications allows investigations on the localisation of blood group substances in cells and tissues from histologic slides (PEDAL and HÜLLE 1984; LÖTTERLE and HEINE 1986), hairs (PÖTSCH-SCHNEIDER et al.1986) and body fluids (BRINKMANN et al. 1986). We applied the APAAP technique for two reasons:
1) There are versatile possibilities of specimen colouring.
2) The inhibition of endogenous peroxidase by hydrogen peroxide can be omitted.

MATERIALS AND METHODS

Specimen preparation: Fingernail specimen with adherent nail-bed were taken from autoptic material of 8 corpses with blood groups A, AB, B and O. Frozen 4-5 µ sections were submerged and floated carefully during each working step.Portions of fingernails were contaminated with blood and buccal cells, respectively. The adherent cells were fixed by aceton/methanol(1:1). A limited number of nail samples were prepared with blood/buccal cell mixtures in various ABO constellations. For saving sera and reagents we had to use defined stain material. Blood grouping of mixed material requires a directed regimen of repeated incubations with anti-H, anti-A and anti-B antibodies. The microtome-section technique, however, permits the production of a sufficient number of specimen for smallest stain samples.

Furthermore, fingernail fragments of 8 volunteers were embedded in a biocomponent adhesive (UHU plus schnellfest(R)) according to GRIEVE and KOTOWSKI (1986) and cut in the usual microtome technique.

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APAAP staining: The following commercially available reagents were used:

Monoclonal mouse, anti-A, anti-B, anti-H (Seraclone) - Biotest
Rabbit anti-mouse immunoglobulins - Dakopatts
Alkaline phosphatase-anti-alkaline phosphatase complex(mouse) - Dakopatts
Naphtol AS-MX phosphate - Serva
Fast Red TR - Serva,Chemapol
Fast Blue RR - Chemapol

All specimen were washed in Tris-buffered saline (0.05 M, pH 7.6) before each reaction stage. Monoclonal anti-A,anti-B and anti-H were diluted 1:50 and incubated with the samples in a moist chamber for 72h at +4°C. The incubation with the linking rabbit anti-mouse antibody (dilution 1:25) was carried out overnight at +4°C. The APAAP complex (dilution 1:300) were coupled for 2 hours at room temperature. The visualisation of the APAAP complex was achieved by treating the reactants with the appropriate dye for 30 min.. Red colouring were intensive when positive. The blue dye, however, allowed a better distinction of positive red blood cells from negative erythrocytes with their individual reddish colour.

Controls: Positive and negative controls must be processed along with all unknown specimen. A nonspecific staining due to endogenous alkaline phosphatase or protein binding was excluded by blank controls with buffer. For testing the specifity of the primary ABH antibody we controlled the lack of reactivity of incompatible antibodies in each case, e.g. anti-B against group A etc.. The reliable and specific tissue staining of epidermis belonging to specimen has proved an excellent tool of control for ABH determination in nail-keratin.

RESULTS AND DISCUSSION

Frozen sections: Frozen slides are the specimen of choice. The adherent nail-bed epidermis allows a clear-cut determination of ABO constellations in the stratum germinativum. The validity of ABO grouping decreases in our material in the known rank order: B> A> H> AB. As expected, we found a weak background colouring of nails after anti-H incubation. However, the striped and dotted colour pattern of keratin tissue enabled the specific staining reaction to be recognized. Thorough inspection of the whole specimen is necessary.

Adhesive embedded sections: In practice the investigator is confronted with small nail-fragment samples, which have to be prepared as slides in a suitable embedding medium. In accordance with GRIEVE and KOTOWSKI (1986) we found that the adhesive UHU meets all criterias for our purposes: It allows easy sectioning without being so hard as to brittle and chemically it is inert. The determination of A and B substances was also succesful in all samples. Nevertheless, nonspecific staining may occur at the border to the embedding medium.

Cell contaminated sections: The blood grouping of red blood and buccal cells sticking to the nail surface revealed encouraging results. To illustrate this, we were able for instance to distinguish between group A erythrocytes or group B buccal cells on the one hand and group O of the layer of nail on the other hand. We have to stress, that in general more complicated constellations such as group AB materials on fingernails of group O cannot result in a diagnosis without restrictions.

In general, positive reactions are weak in comparison with other tissue material because of the low concentration of ABH antigens in nails. The drawbacks of all immunoenzyme methods are their numerous reaction stages with extended incubation times. In our experiences the incubation of nail-keratin with primary antibody should not be reduced below 48 hours. Considering the total of working steps the test needs more than 3 days. As a rule, we have to expect an additional damage of stain material from the scene. Therefore, the prior storage time for red blood and buccal cells should not exceed a few days only. This limitations does not concern pure fingernail samples.

CONCLUSIONS

1) APAAP staining is a proper method for demonstrating blood group antigens in fingernails from groove to margin.

2) Frozen sections as well as smallest specimen embedded in a suitable adhesive are applicable for staining procedures.

3) Using fresh prepared artificial stains, blood group constellations of red blood cells and/or buccal cells adherent on the surface of fingernails may be distinguished from the nail matrix.

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